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This number of the Journal of the Optical Society of America is composed of papers in the field of microscopy, many of these being based on papers presented at the October, 1949, meeting of the Optical Society held in Buffalo, New York. The symposium on microscopy arranged at that meeting covered many of the important developments in such fields as phase contrast, reflection optics, and electron microscopy. The symposium in Buffalo nearly coincided with the completion of the first century of progress in American microscope construction, dating from the first Spencer model of 1838.

Editor

Electrons *vs.* Photons: A Comparison of Microscopes*

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A comparison of the transmission-type electron microscope with the similar light microscope has to include considerations of the differences in the nature of the carrier of radiant energy and of the optical media used for building up the optical systems. Although electron optics is of relatively recent origin, fairly good agreement of the experimental limits with the theoretically expected ones has been obtained. After a discussion of existing discrepancies, proposals and attempts for improving the performance of the electron microscope are outlined.

A COMPARISON of the electron microscope with the light microscope could be made from different view points. One way of presenting such a comparison would be on a statistical basis—comparing the number of instruments existing. The total number of electron microscopes is insignificant as compared to the number of light microscopes; whereas there are about 500 electron microscopes around the world, the number of light microscopes can be estimated probably as being in the hundreds of thousands. It is doubtful if any conclusions or predictions can be made from such a comparison. It is probable that as light microscopes proved to be extremely useful, so will electron microscopes become more and more popular; and with cheaper production methods, they will find their way into the average laboratory. If they do, they will not be used as competitors to the light microscopes. It is hoped that the present discussion will show that the two instruments have to be coexistent and complementary.

A much more interesting point of view for the comparison of the two instruments is the physical one. Such a comparison resolves itself into essentially three categories. First, a comparison of operating principles, second, the differences in instrument design, which are derived directly from the operating principles, and third, a comparison of performance of the two types of instruments. For simplicity, our discussion will be limited essentially to the transmitting type of instrument.

From the point of view of operating principles again, our considerations must be divided into three subcategories. Every optical system needs some kind of carrier of radiant energy. First of all, therefore, the properties of the energy carriers, in one case the photons, and in the other the electrons (and/or any other charged particles) should be discussed. The carrier of radiant energy cannot be used without some kind of refractive or reflective medium, so, second, the properties of such media should be considered. Last, there must be a specimen and the properties of the specimen, or to be more precise, the interaction of the radiant energy with the material of the specimen, should be studied to elucidate the difference in behavior and operating principles of the two types of instruments.

Table I lists the properties of the two most important

* Presented as an invited paper at the Buffalo meeting of the Optical Society of America, October 1949.

TABLE I. Properties of the carriers of radiant energy.

	Photon	Electron
Charge	0	$-e$
Mass	$h\nu/c^2$	$m=m_0[1-(v^2/c^2)]^{-\frac{1}{2}}$
Rest mass	0	m_0
Momentum $p=$	$h\nu/c$	mv
Energy $W=$	$h\nu=cp$	$mv^2/2=c(m^2c^2+p^2)^{\frac{1}{2}}$
Wave-length $\lambda=$	$c/n\nu$	h/mv
Group velocity	$c\frac{d\nu}{d(n\nu)}$	$v=\frac{c(h\nu+e\phi)^2-m_0^2c^4}{h\nu+e\phi}$
Phase velocity	$c'=\lambda\nu=c/n$	$\frac{c^2}{v}=\frac{e\phi}{m_0v}(1-v^2/c^2)^{\frac{1}{2}}$
Wave equation	Solution real	Complex
Statistics	Bose	Fermi

TABLE II. Properties of optical media.

	Photon	Electron
Index of refraction	$n=\frac{c}{c'}=\frac{c}{\lambda\nu}$	$n=\frac{\lambda_0}{\lambda}=\frac{m}{m_0}\frac{v}{c}$
Shape of refractive elements	Arbitrary	Limited by Laplace equation
Optical components	Static	Dynamic

types of carriers of radiant energy—that of the photon and that of the electron. The fact that an electron carries a charge, whereas a photon does not, introduces a very important difference between the two types of microscopes. Due to the charge carried, there exists a mutual repulsion of the individual particles resulting in a tendency of the electron beam to spread. Although, in the case of microscopy, operation is usually at a very low beam intensity (where such mutual repulsion is rather weak) it cannot be neglected entirely and it plays a considerably more important role in the design of other electron optical devices.

There is a difference between mass and rest mass of the two carriers. Even at zero velocity, the electron is a massive particle and the interaction between electron and matter will have to be different from that of light. Both the energy and the momentum of the electron are continuously variable and can be changed arbitrarily. The remaining properties described in Table I will be enumerated when the interaction of the carriers of radiant energy with matter is discussed.

The differences between the refractive and reflective media of optics must be considered. Table II lists the differences in the indices of refraction and of the shapes of refractive elements. The most important consequence of this last comparison is that, whereas the refractive index of light optics is usually contained between the limits of $n=1$ to 2, the limits of electron optics are incomparably wider. Variations of the refractive indices in the ratio of 1000 to 1 are permissible and are often used. Furthermore, whereas the refractive media of light optics are practically immutable, the refractive media of electron optics are continuously variable. As regards the shape of refractive elements, light optics has the definite advantage. Although at a first view, the light optician may look with envy at the simplicity with which the change in shape of an electron optical lens can be accomplished, he may be less envious when looking at the limits imposed on this shape. In light optics, there are spherical or aspherical lens shapes, a similar variety of mirrors, and any other type of refractive elements can be manufactured; although in some cases, the manufacturing process may be rather complicated. No such freedom is given to the designer of electron optical instruments. If he has a given field distribution along the optical axis of a lens, his whole field distribution in space is automatically prescribed by the Laplace equation. It is very hard to get around this limitation; special tricks are needed to avoid some of its dire consequences. Furthermore, whereas light optics deals rather infrequently with media of continuously varying indices of refraction, in electron optics this is the common case. Due to the fact, however, that light optics deals with fixed refractive elements (i.e., lenses, mirrors, or prisms having fixed shapes) the importance of dynamic optical elements is negligible in light optics whereas it is increasingly greater in electron optics.

The difference in interaction between the carriers of radiating energy and matter is a difficult question, because, in spite of 300 years of development in light optics, we are ignorant of *all* the details of such interaction in light optics. After 20 years of electron optical development, our greater degree of ignorance in electron optics may be pardonable. It is noted in Table I that both types of carriers obey wave equations of their own, and the solution of such wave equations in the case of light has a real solution whereas the amplitude of the electron wave is complex. Furthermore, there is no single equation existing for the two optics which could satisfy the conditions with different constants for the two cases. From the existence, however, of wave equations, it may be inferred that one of the primary phenomena governing the image formation in both cases, is diffraction. Thus Abbe's theory (with the usual limitations) applies in both cases and the resolving power can be derived from quite similar considerations. The next item in Table I, the difference in the statistics applied to the individual carriers, denotes a marked difference in behavior. If an ideal specimen which does not absorb is observed in monochromatic light, the whole image formation can be described in terms of refraction and of phase differences. If no energy of the radiant beam is transferred to the substance of the specimen, the energy of the transmitted beam will remain unchanged. No such case is possible for electron optics. Without exception, a substantial proportion of an electron beam passing through matter will be incoherently scattered with resulting energy loss to the specimen. In the case of specimens composed of atoms with low atomic number, the proportion of incoherent

scattering (i.e., of inelastic scattering) will be preponderant.[1] If there is interaction of light with matter, it will have more often the character of a reversible reaction with practically no change in the entropy of the system; whereas in cases of interaction of electrons with matter, the resulting changes will more often have the character of an irreversible reaction with a resulting change in the entropy. The results are twofold. There is a change in the character of the beam and, due to the energy loss, there is a resulting change of the specimen.

Besides these differences between the two types of optics, there are some striking analogies. There is the remarkable analogy between Fermat's principle, governing the path of light in a refractive medium, and the principle of least action which applies to the trajectories of charged particles in fields of forces. As a result of this analogy, a very satisfactory system of geometrical electron optics can be described and ray-tracing methods have considerable success in both types of optics. On the other hand, the analogies go so far as to provide electron optics with all the known aberrations of light optics (plus a few others). Some of the aberrations such as chromatic aberration can be partially corrected by operating the electronic instruments with a strictly monochromatic beam, meaning, in this case, an electron beam of uniform velocity. Unfortunately, the remedies are not as easy in the case of some other aberrations, which will be discussed briefly in a later part of this paper.[2]

The vast number of differences between the two types of optics reflect themselves in the difference in instrument design, for example, the more electrical character of the electron microscope as compared to the design of a light microscope. Electron microscopes must be operated in a good vacuum although there are light optical instruments which require the use of a reasonably good vacuum for the very far ultraviolet.

Due to the flexibility of electron optical elements, it is more common to replace mechanical focusing by an electrical one, resulting in a fixed position of the object and a variation of the focal distances of the lenses, although electrostatic electron microscopes sometimes revert to a mechanical displacement of the object before the objective. Due to the previously discussed necessity of monochromatic illumination, great care must be taken in the design of the illuminating systems. They are always an integral part of the microscope, operated in the same vacuum, and rigidly connected to the remainder of the optical system. This, however, is not a very striking difference, because there is a marked tendency in light optics to make the light source an integral part of the microscope. An important difference is due to the lens characteristics. At present lenses in electron optics have focal distances of about the same order of magnitude as lenses of light optics. Figure 1 shows the focal distances of some magnetic lenses as a function of the energy of the incident beam, and indicates that it is rather hard to get below focal distances of 1 mm. The commercially available electron microscopes in different countries have lenses with focal distances of 2 to 5 mm.[3]

Originally, electron microscope design followed the pattern set by light microscope design, and compound microscopes with two stages of magnification were used. On the other hand, electron microscopes, due to their increased resolving power, have to be operated usually at higher magnifications than light microscopes. The result was a considerable increase in length of the instruments as compared to light microscopes. In recent years, however, the tendency is away from a two-stage magnification and toward a three-stage magnification.[4]

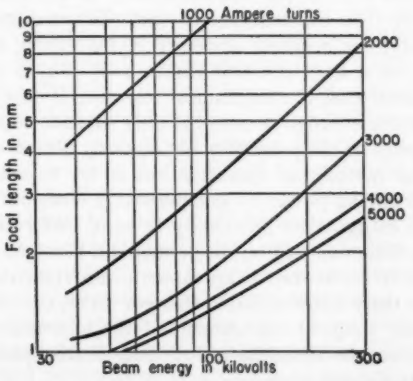

FIG. 1. Focal distances of a magnetic lens as a function of the energy of the electron beam.

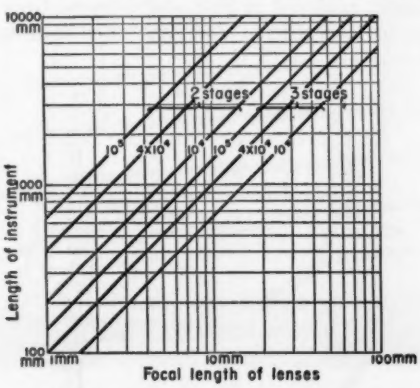

FIG. 2. Instrument length *vs.* focal distance for different magnifications.

[1] L. Marton and L. I. Schiff, J. App. Phys. 12, 759 (1941).

[2] V. E. Cosslett, *Electron Optics* (Oxford University Press, New York, 1946); D. Gabor, *The Electron Microscope* (Chemical Publishing Company, Inc., Brooklyn, New York, 1948); V. K. Zworykin *et al.*, *Electron Optics and the Electron Microscope* (John Wiley and Sons, Inc., New York, 1945); L. Marton, *Reports on Progress in Physics* (The Physical Society, London, 1946), Vol. 10, p. 204.

[3] E. Ruska, Archiv. f. Elektrotech. 38, 102 (1944).

[4] L. Marton, J. App. Phys. 16, 131 (1945); J. B. Lepoole, Philips Tech. Rev. 9, No. 2 (1947); Haine, Page, and Garfitt, J. App. Phys. 21, 173 (1950).

Figure 2 illustrates the reduction of instrument length. The reduced length of the instruments means greater mechanical stability and greater freedom from external influences. Besides such rather obvious advantages, there is another advantage. Ever since the conception of the electron microscope, attempts have been made to use such instruments alternately as an electron microscope and as an electron diffraction camera. Such a conversion from one type of operation to the other is rather clumsy in the case of a two-stage instrument where the conversion means either a mechanical displacement of the specimen or a mechanical displacement of the projector lens pole pieces. In a three-stage instrument, the conversion can be made by changing the refractive power of one lens as indicated in Fig. 3, and by inserting a field limiting aperture in front of the intermediate lens.

In the external appearance of instruments there is a greater influence to concessions of the popular taste, for instruments nowadays have to be "streamlined." Such concessions however are not new, as anybody can judge who has seen old light microscopes decorated with fancy curleques. The instrument designer always tried to make the instrument pleasing to the eye as well as useful, and it is not his fault if what was pleasing yesterday is not pleasing today.

Next there is the comparison of the performances of the two types of instruments. Previous mention was made of the validity of Abbe's views for both types of optics. At first, one would be inclined to expect that a system operating with radiation having a hundred thousand times shorter wave-length than light would have that much higher resolving power. The presence of aberration or, to be more correct, the lack of knowledge to correct the aberrations of electron optical systems, restricts the operation of electron microscopes at about 1000 times smaller numerical apertures than the highest achieved in light microscopes. Even with the present aberrations, however, one would expect a

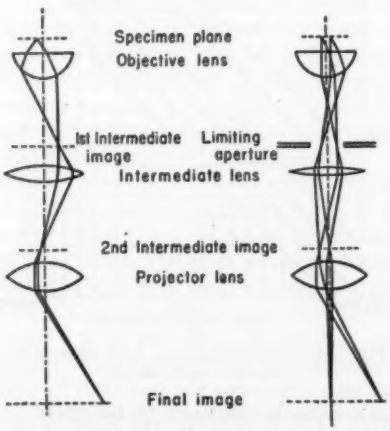

FIG. 3. Ray diagram, (a) for high magnification image formation, (b) for diffraction experiment.

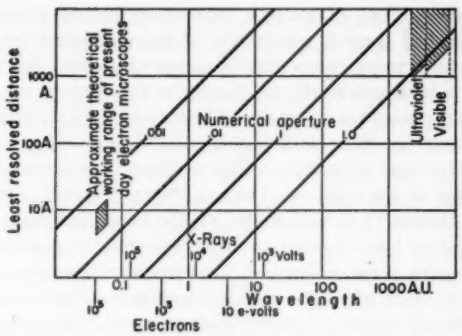

FIG. 4. Least resolved distance vs. wave-length for different numerical apertures.

least resolved distance of about 5 to 8A in contrast to about 1000A for the ultraviolet microscope (Fig. 4). There is a lack of agreement among research workers in electron microscopy as in light microscopy as to what constitute the proper criteria for the limit of resolution. Depending on the criteria used, the best experimental values can be listed as ranging from 10 to 15A. Why the discrepancy of a factor of two between the theoretical prediction and the best experimental values exists is hard to explain. Spherical aberration of course is always present. The value of the aberration constant has been measured repeatedly and the theoretical limit of resolution given above was calculated according to the measured values of that aberration. For some time it seemed as if an inherent astigmatism of lenses would be the main limiting factor. It was recognized that astigmatism can be due to mechanical imperfections of the elements composing the lens, such as the accuracy of machining, but also due to the inhomogeneity of the materials used for the construction of such components. Incidentally, the required accuracy of machining is very high; for a least resolved distance of 20A, it can be calculated that the mechanical tolerance is only 0.02μ. Under these circumstances the only solution seems not to depend on the possibility of achieving ultimate accuracy by mechanical operation but to try to reach the best resolving power by compensating elements which reduce astigmatism practically to zero. Unfortunately, such a reduction may not be permanent. Electron microscopes are somewhat temperamental instruments, subject to daily uncontrollable changes which may require not only frequent realignment of the instrument, but sometimes also repetition of the compensating procedure for zero astigmatism.

If the values of the least resolved distances are used, the electron microscope is certainly useful at dimensions exceeding the limit of the light microscope by about a factor of 100. The question remains "Is the electron microscope also useful in the range where light microscopes are usually employed?" In most cases the answer is negative. When the dimensions of a specimen are large enough to be observed in a light microscope,

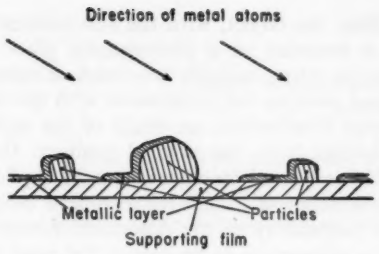

FIG. 5. Principle of shadow casting.

usually the electron microscope does not give any added information. This is therefore a clear indication that the two instruments do not overlap, but have to be used concurrently.

Considerable progress has been made also in the last few years in emphasizing contrast in the electron optical image. Part of this type of development is due to improved methods in preparing our specimens. In recent years, a very beautiful method was developed for that purpose, consisting of evaporating metals in vacuum on to the surface of a specimen.[5] If such an evaporation is done at an angle of, for example, 5° in a good vacuum, elongated shadows of the observed particles are produced, and at the opposite side of the shadow, a thin deposit of metal will be formed on such particles (Fig. 5). Such a shadow casting method greatly emphasizes the existing contrast in the image and enables the recognition of details below the depth resolving power of the instrument.

Phase contrast phenomena are far from negligible in the image formation of the electron microscope, although as yet there has not been much success in producing phase contrast at will. The difficulties are essentially of instrumental nature; it is very hard to make a phase plate of, for example, 135A thickness and with a perfectly centered aperture of about 1μ in diameter. If such a phase plate is made, there is no assurance that the phase plate will stay unchanged due to the various interactions between the beam and the specimen, between the beam and the molecules of the extremely rarefied gas atmosphere in the instruments, between beam and the materials composing the instrument walls, and other effects. Due to all these interactions, there is always a continuous modification of the phase plate during the time of observation.

One type of operation of the electron microscope has been neglected up to now in this discussion. That is the dark field observation. In contrast to the light microscope, dark field illumination does not seem to offer many advantages because the resolving power of the instrument seems to be reduced as compared to bright field observation. The reason for the loss of the resolving power is not entirely clear. There are definite indications that the loss of resolving power exists only in case of incoherent scattering, and probably we can benefit from the full resolving power from specimens giving predominantly coherent scattering.[6] Instead of a detailed discussion of these phenomena, it may be preferable to describe briefly a new application of electron microscopes which has no direct parallel in light microscopy.

It was proved in dark field illumination, that an electron optical reproduction of the light optical schlieren effect is possible. However where light optics views variation of the index of refraction, electron optics looks at fields (either electrostatic or magnetic). Extension of such a method enables, therefore, the electron optician to map electrostatic or magnetic fields which are, due to their dimensions, inaccessible to other types of measurements. A detailed description of these methods appeared elsewhere.[7] The inclusion of these remarks about the method and this comparison of microscopes is done, however, to point out to what varying tasks electron microscopes can be put. Figure 6 shows how extremely small fields provided they are not extremely weak (such as fields of individual ferromagnetic or ferroelectric domains) can be observed in an electron microscope.

A comparison of this kind would not be complete without some outline as to future developments. Bernal, in a recent paper,[8] suggested that the advent of the electron microscope was highly beneficial to the light microscope because it stimulated thinking toward improved forms of that instrument. The reverse is also true. Since phase contrast has been accepted by light microscopists, the introduction of phase contrast in electron microscopes has been considered. The electron microscopist follows with great interest the discussions

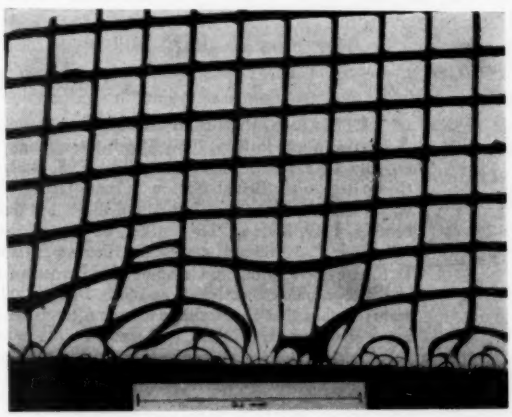

FIG. 6. Pattern representing field distribution from single ferromagnetic domains (courtesy of *J. App. Phys.*).

[5] R. D. Williams and R. W. G. Wyckoff, J. App. Phys. **17**, 23 (1946); R. W. G. Wyckoff, *Electron Microscopy* (Interscience Publishers, Inc., New York, 1949).

[6] C. E. Hall, J. App. Phys. **19**, 198, 271 (1948).

[7] L. Marton and S. H. Lachenbruch, J. App. Phys. **20**, 1171 (1949).

[8] J. D. Bernal, Proc. Phys. Soc. (London) **A62**. 537 (1949).

on the nature of image formation, on resolving power, etc. Attempts are being made to find ways and means for improving the performance of the electron microscope and, in principle, there are several ways in which that can be done. The most obvious is the improvement of lenses. Due to the fact that spherical aberration is the most important limiting factor, there are serious attempts in different laboratories toward reduction of the spherical aberration.[9] Unfortunately, the least resolved distance is proportional to the fourth root of the spherical aberration constant, meaning that if the least resolved distance were reduced by a factor of 10, the spherical aberration would have to be reduced by a factor of 10,000. That approach does not look too hopeful. Another type of approach would be to reduce even more the wave-length. The proton microscope would be a possible answer to that question but the interaction between a specimen and a particle with almost 2000 times greater mass than the electron will limit the application of such an instrument to a rather restricted class of objects. A third approach to the problem is called "microscopy by reconstructed wave fronts." This type of microscopy is essentially a two-step process. The object is first illuminated with a coherent monochromatic wave. The diffraction pattern resulting from the interference of the coherent secondary wave, issuing from the object, with the still coherent background is recorded on a photographic plate. If the photographic plate, suitably processed, is replaced in the original position and illuminated with the coherent background illumination, an image of the object will appear behind it in the original position. Gabor in England[10] has proved his principle to be applicable to the reconstruction of a light microscopical image. The Research Laboratory of the Associated Electrical Industries is attempting to reproduce the same kind of image formation with electrons.[11] In this work, only the first step, that is, the decomposition of the image into a diffraction pattern, is done with electrons; whereas the second step, the synthesis of the final image, is done with light. In the first step as many as 14 fringes were produced, thus giving a fair chance for completing the process. The difficulties, however, are very serious because the image synthesizer must have the same aberrations as the analyzing system. In principle, this type of image formation should be able to go considerably beyond the limits imposed by the conventional type of electron microscope perhaps as far as 0.5A. It remains, however, to be seen to what extent the enormous technical difficulties of the new process can be overcome.

[9] O. Scherzer, Optik **2**, 114 (1947).

[10] D. Gabor, Proc. Roy. Soc. (London) **A197**, 454 (1949).

[11] Private communication from Dr. T. E. Allibone, and M. E. Haine, Assoc. Elec. Ind. Ltd., Res. Labs.

Optics in London in July

OPTICAL scientists from various parts of the world will convene at Imperial College in London during two weeks in July, 1950. The International Commission of Optics will hold its second formal meeting on July 17–21; U. S. delegates will be S. S. Ballard, I. C. Gardner, and R. Kingslake. The Optical Sub-Committee of the British National Committee for Physics has organized a conference on optical instruments, which will also be held at Imperial College, but during the period July 19–26, as was announced in the December, 1949, issue of this Journal. U. S. scientists who have been invited to present papers include the following: C. L. Bausch and G. R. Harrison, "The Design and Production of Echelles for Spectroscopy"; D. S. Grey, "A Family of Catadioptric Microscope Objectives"; R. Kingslake, "Some Recent Developments in Photographic Objectives"; and S. S. Ballard, "The Present State of Spectrophotometry in the U. S." It seems certain that there will be a large attendance of British and continental optical people, and a full program of meetings, exhibits, and social events is being planned. American scientists who may be visiting London this summer are invited to attend these meetings.

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Microscope Optics*

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A brief history of the major advances in microscope optical designs precedes a description of problems facing the designer and the manufacturer. Recent developments to expand the usefulness of the microscope and to make its effectiveness more nearly approach theory are discussed. Attention is given to the various forms the instrument takes when applied to special fields.

INTRODUCTION

WHATEVER the writer can say about the subject of the microscope is based upon contact with microscope problems over the past thirty years. When first introduced to the subject, microscope objectives were literally made by hand. Skilled lens mounters made the mounts, which were to hold the tiny lens components of these microscope objectives, out of brass or german silver rod. These were made on rather simple lathes and the mounter carried through to completion only 6 objectives of any one focal length at a time. All objectives consisted of a front and a back, and the back, which was interchangeable on all, was made on a normal lathe. The front was made on a micro lathe and held the lenses. The first lens was mounted directly in this piece, and the second lens was mounted in a cell which screwed onto the first. The third one was in a cap-like mount, which threaded on the first lens mount and covered up the intervening lenses. The six objectives were inspected and kept together until they were approved for sale. They always had to be "set," which meant that a sensitive air space was adjusted to correct for errors of spherical aberration. By observing an Abbe test plate, the adjuster could tell very easily whether the space was to be increased or decreased, and he could indicate rather precisely how much change should be made. The mounter then made the change by facing off metal or adding on by burnishing up the metal and recutting a true shoulder. A large percentage of objectives only required one such operation, but there always were those which required several adjustments before they were good. The mounters never made more than a few mounts at a time, knowing that as the lathe and the chucks wore, the work could not be put back on the chucks without errors.

It was not long after my introduction into this work that precision lathes and diamond turning operations became available. If lens cells could be turned to accurate diameters and put in well-centered collets, many lenses could be mounted at a time and later assembled in a sleeve mounting. This process was worked out to such an extent that the different components of objectives could be mounted by different operators, and then brought together to produce well-centered and well-adjusted objectives. Figure 1 shows the present method of mounting the lenses in cells which fit snugly in the sleeve mounting. The group of lenses are held in position by the threaded sleeve and locking nut, which may be used to adjust the position of lenses with respect to the shoulder to make them parfocal.

During these thirty years of close contact with the microscope, many other problems have faced both the designer and the manufacturer. The early objective was a work of art, while the present objective is a work of precision. Thirty years ago, glass was a problem. It was made in small quantities and with only limited control of its optical properties. The designer was kept busy changing the formulas as batches of glass were used up. It was a serious problem to balance out the stock of lenses made from one formula, and to keep the lenses from two different formulas from being mixed. In most cases, the lenses from two formulas looked exactly alike to the eye. If a wrong lens found its way in an objective, it was usually recognized by a badly colored image. Sometimes wrong glass would have less effect and, consequently, the setter had to be alert to recognize wrong glass from errors in manufacture. Great progress has been made to hold the optical properties of glass, so that a formula rarely needs to be changed and only when the designer has made an improvement in the optical quality. Methods, such as those described by Cruickshank,[1] have aided the engineer in setting up tolerances on thicknesses, spacings, curvature and glass, so that all possible combinations which can be tolerated can be predicted and made use of in making objectives. Epstein,[2] and later Wooters,[3] have studied the effects of centration on central and marginal coma and recommend where the greatest accuracy of cementing, truing, edging and mounting must be applied.

MICROSCOPE OBJECTIVES

The microscope objective has been made in three different types, namely the achromatic, semi-apochromatic (sometimes called fluorite) and the apochromatic. The achromatic type can be subdivided into low power, medium power, high dry and oil immersion forms. The low powers are made with a single doublet when the

* Presented as an invited paper at the Buffalo meeting of the Optical Society of America, October 1949.

[1] F. D. Cruickshank, Proc. Phys. Soc. 57, 350, 362, 419, 426, 430 (1945).
[2] L. I. Epstein, J. Opt. Soc. Am. 39, 847 (1949).
[3] G. Wooters, J. Opt. Soc. Am. 39, 1059A (1949).

numerical aperture is approximately 0.10 or less, and with two achromatic doublets when the numerical aperture is about 0.25. The medium and high dry forms are usually made with a strong unachromatic front lens and two achromatic doublets, while the oil immersion consists of a strong hemisphere and meniscus lens, which are highly undercorrected followed by two overcorrecting achromatic doublets. Careful examination of the performance of these objectives indicates that the low power objectives (0.10 N.A. and less) have excellent central definition quite free from annoying colors even when used with high power eyepieces. The low power objectives of numerical aperture 0.25 are not quite so good when tested with high power eyepieces. They will display some little secondary spectrum. This is reduced appreciably if the objective is made apochromatic. Figure 2 shows the construction of the 16 mm achromatic and apochromatic objectives and their corresponding aberration curves. If we think of secondary spectrum as either the sum of the sag of D below a straight line drawn from C to F and the height of G' above this extended line, or the height of the greatest of these if they are on the same side of the straight line, then the secondary spectra for the 16 mm achromatic and apochromatic objectives are 2.95 mm and 0.05 mm respectively. These low power objectives are all corrected well within the Rayleigh limit of one-quarter wave difference in optical path. When we come to the medium and high power objectives, experience has shown that this criterion cannot always be met.

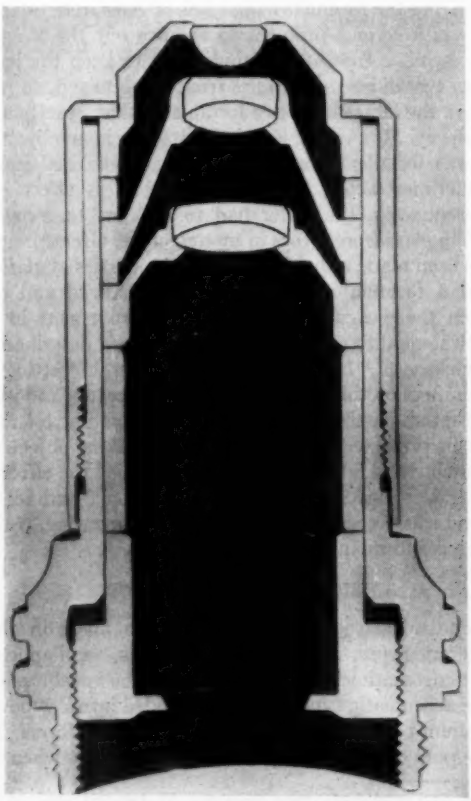


FIG. 1. Cross-sectional view of Bausch & Lomb 4 mm, 0.65 N.A. objective shows two achromats and one hemisphere, each mounted in a cell which slides in a sleeve.

As an example, in the 4 mm, 0.85 N.A. achromatic objective, if the one-quarter wave-length tolerance is put in terms of longitudinal spherical aberration and zonal aberration, for light having wave-length of 5893 A, the spherical aberration should not exceed 5.5 mm, and the zonal aberration 8.2 mm. Yet, the computed aberrations for the best balanced system are 15 mm and 23 mm respectively. Another 4 mm objective, the 0.65 N.A. achromat, however, could have 11 mm spherical aberration and 16.6 mm zonal aberration. This objective actually has about 3 mm spherical aberration and 5 mm zonal aberration. It is corrected to about one-third of a Rayleigh limit. When these objectives are adjusted to give the least amount of spherical aberration by observation of a pinhole, they will, in an interferometer test, show about the amount of aberration predicted by the Rayleigh criterion. These two different objectives having the same magnification are made for different purposes. The objective with 0.65 N.A. is made for use by the medical student as an all-purpose, high power, dry objective. It is made with a long free working distance of 0.7 mm between an 0.18 mm cover glass and its front mount, so that it can be used through thick cover glasses, such as are encountered in blood counting techniques. The objective with 0.85 N.A. is made for use where greater resolution is required, but without the inconvenience of immersion. From the designer's standpoint, it is important to interpret what the effect of the spherical aberration is in the formation of images. If a lens is corrected for spherical and zonal aberrations within Rayleigh limits, which is expressed as a quarter wave difference in optical path length for all rays from the object to the image, it should give an image of a point of light in which the intensities in its vicinity follow the pattern described by Airy. If there are aberrations greater than this, then more light falls in the rings of the Airy disk and lack of contrast in images of detail and dark areas should result. Resolution will not fall down, however, until the aberrations are so great that the quantity of light falling in the first ring of the Airy disk approaches that concentrated in the central image.

RESOLVING POWER TESTS

It seemed worthwhile to take some pictures to show the resolution of the two 4 mm achromatic objectives. Diatoms afford a variety of structures suitable as objects to use for this work, and pleurosigma angulatum was selected as a diatom which could not be resolved in central illumination with the 4 mm, 0.65 N.A. objective, but could easily be resolved with the 0.85 N.A. ob-

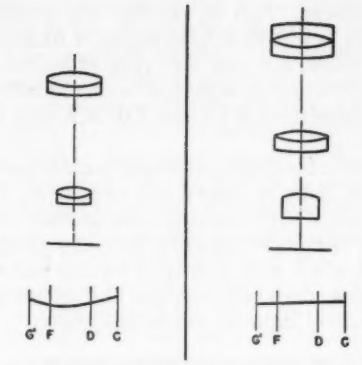


FIG. 2. Optical construction of Bausch & Lomb 16 mm achromat on left, and Bausch & Lomb 16 mm apochromat on right. Curves show axial chromatic aberration.

jective. Three pictures were made of this diatom with the two objectives. All pictures were made using a 20× Hyperplane eyepiece, at a magnification of 860×. Panchromatic film was used and was developed 4 minutes at 20°C in DK-60a to give normal contrast. A ribbon filament lamp with an interference film filter having maximum transmission at 4960A served as illuminator. The microscope was equipped with an aplanatic condenser and a decenterable rotating iris diaphragm. Figure 3a was made using the 4 mm, 0.85 N.A. objective with central illumination. The size of the iris diaphragm was adjusted to give an illuminating aperture of 0.35 N.A. The adjustment was made by looking at the back aperture of the objective and noting when the diffracted images of the iris diaphragm became tangent to the central direct image. This picture clearly shows the dot structure of the diatom, but it is lacking in contrast. Figure 3b was made using the same objective but with oblique illumination. The substage diaphragm was decentered so that it gave an oblique beam 90° to the axis of the diatom. The obliquity was such as to distribute the direct and the diffracted images symmetrically about the axis of the objective. The effective numerical aperture was 0.23. This picture has considerably more contrast than the first, and this can be caused for two reasons: First, the illuminating cone has been reduced from 0.35 to 0.23 N.A., and second, it is oblique to the axis of the objective, so that it and the diffraction images which go to form the final image of the object occupy only one zone, which in itself is corrected for spherical aberration within the tolerance. The first picture used all of the aperture of the objective which has about 3 times the Rayleigh limit. The third picture, Fig. 3c, was taken using the 0.65 N.A. objective and the diaphragm further reduced to 0.10 N.A., but left at the same obliquity as in Fig. 3b. The aperture was reduced in order to have all of it contained within the aperture of the objective. This picture is quite comparable with that of Fig. 3b, as should be expected. Incidentally, since these pictures show the diatom resolved, the negatives were measured and the space from one line of dots to the next indicates that in the diatom this separation is 0.00052 mm. From a crude measurement of the N.A. of the off-axis illuminating beam and the known wavelength of light used, the separation should be 0.00048 mm, which is in close agreement with theory.

Observation with the 4 mm, 0.85 objective indicates considerable secondary color in the very center of the field. This is to be expected, since the longitudinal chromatic aberration, $F-C$, is 10 mm. The 4 mm apochromatic objective has only 2.0 mm chromatic aberration and thus can be expected to give much less color than the achromatic form. Since there is so much difference in color between the achromatic and the apochromatic objectives of this focal length, one would expect a proportionate gain in a semi-apochromat. This is the case in the 4 mm semi-apochromat. The construction of these three objectives and their aberration curves are shown in Fig. 4. The same argument can be given for the oil immersion objective, but since the focal length is shorter, and the aperture greater, the color appears even worse. The comparison of the forms, achromatic and apochromatic, is shown in Fig. 5.

These basic types and forms of objectives have remained practically unchanged during my 30 years of experience. In this time, some alteration of objectives has been made. For the most part, they are for special purposes or special applications on specific instruments. A few examples may be cited, such as the 32 mm objective used extensively with vertical illumination on opaque metal objects. When the object was diffusely reflecting, the performance was good, but when objects

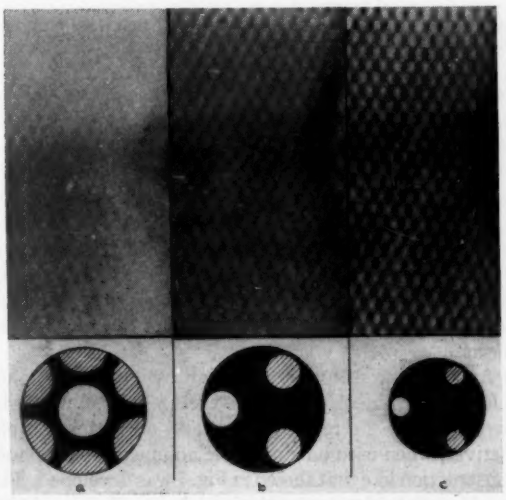


FIG. 3. Photomicrographs of pleurosigma angulatum and appearance of back aperture of objectives. (a) 4 mm, 0.85 N.A. objective with central illumination, (b) with oblique illumination, and (c) 4 mm, 0.65 N.A. objective with oblique illumination; clear areas represent aperture and position of illumination pencil; shaded areas represent position of diffraction pencils passing through the objective.

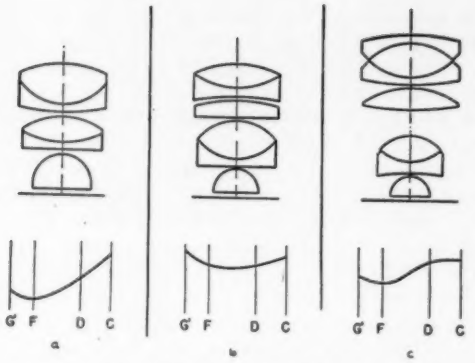


FIG. 4. Optical construction and chromatic aberration curves of (a) 4 mm, 0.85 N.A. achromat, (b) 4 mm, 0.85 N.A. semi-apochromat, and (c) 4 mm, 0.95 N.A. apochromat.

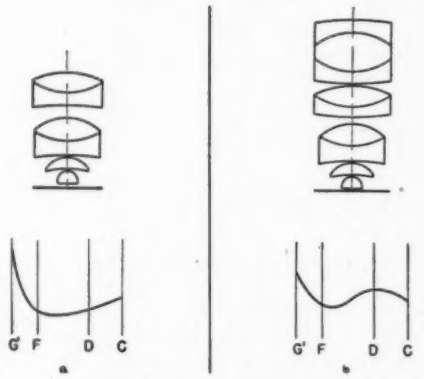


FIG. 5. Optical construction and chromatic aberration curves of (a) 1.8 mm, 1.25 N.A. achromat, and (b) 2 mm, 1.40 N.A. apochromat. Both are oil immersion objectives.

with high polish were examined, illumination fell off badly toward the edge of the field. This was corrected by adding an achromatic lens near the object plane, which acted as a collective directing the diverging pencils of light falling on it back to the objective. A diagram of the path of light incident on the object and reflected from it at the edge of the field is shown in Fig. 6. The specularly reflected beam of light reflected at the edge of the field is 0.06 N.A. in the case of the single achromat, and 0.09 N.A. in the case of the two doublet forms.

Another example was the demand a few years ago for a low power objective, such as the 4×, which would be parfocal with the regular 16, 4, and oil immersion objectives, when used on a multiple nosepiece. A telephoto construction like that shown in Fig. 7 was developed. The focal length is 28 mm, and the initial magnification 3.2×. The mechanical tube length is substantially longer in metallographic microscopes than in biological microscopes. In order to maintain magnifications about equal to those in the biological microscope, and to achieve large flat fields, a series of longer focal length objectives were developed. That is, on this microscope will be found a 24 mm, 0.20 N.A. objective, a 10.25 mm, 0.40 N.A. objective, a 6 mm, 0.65 N.A. objective and two fluorite, oil immersion objectives having focal length and numerical apertures of 4.3 mm, 1.00 N.A. and 2.75 mm, 1.25 N.A.

There have been many attempts made to increase the flatness of field of microscope objectives, but this attempt has always required some sacrifice in central definition. Boegehold[4] showed a series of planachromatic objectives which were developed for this purpose. The photographs which accompanied the reference article show a marked improvement in this respect.

ULTRAVIOLET MICROSCOPY

In addition to requirements of resolution of fine detail, emphasis has recently been put on the spectral absorption differences, due to the chemical compounds making up biological material. This is rapidly extending the use of the microscope into the ultraviolet and infra-red portions of the spectrum. The importance of work in the ultraviolet portion was recognized a great many years ago as one means of increasing resolving power. Today the advantage in using ultraviolet light to distinguish between different biological materials is becoming equally as important as the increase in resolution. In 1903, Dr. Koehler[5] introduced his quartz monochromats for the prime purpose of increasing resolving power. They were constructed of one kind of refracting ma-

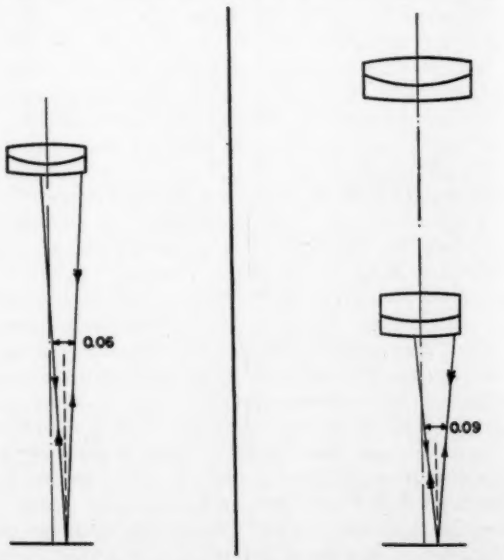


FIG. 6. Light incident on a specularly reflecting object follows the laws of reflection and, if no collective is near the object, the edge of the field will lack brightness. At the edge of the field the N.A. of the reflected beam received by the objective is 0.06 for the single achromat, but 0.09 for the two achromatic objectives.

[4] H. Boegehold, Zeits. f. wiss. Mikroskop. 55, 17 (1938).
[5] A. Koehler, Zeits. f. wiss. Mikroskop, 21, 129 (1904).

terial, namely fused quartz and, as a consequence, were only corrected for one wave-length in the ultraviolet. To use them required focusing by photography. The writer[6] described in 1946 an achromatic objective composed of fused quartz and fluorite for use in a sufficiently broad range in the ultraviolet, so that focusing could be done by using a fluorescent screen. B. K. Johnson[7] described this year a 16 mm, 0.27 N.A. objective constructed of fused quartz and lithium fluoride. He shows pictures made, after focusing in visible green, at wave-length 2537, 2749, 3131, 3650 and 4358, which are reasonably well in focus. Brumberg[8] in 1942 described a two-mirror reflecting objective of 6 mm focal length and 0.50 N.A. Burch[9] in 1947 described two aspheric reflecting objectives, one of 7.5 mm focal length and 0.58 N.A., and the other a 3 mm, 0.65 N.A. The latter could be made 0.98 N.A. by adding a hemisphere. David Grey and Paul Lee described a series of Catadioptric Newtonian and Schwarzschild systems of microscope objectives.[10,11] The writer[12] described before the American Chemical Society in 1948 a 2.8 mm, 0.72 N.A. reflecting objective, which D. Grey of Polaroid Corporation had designed, and which was made at Bausch & Lomb Optical Company. In order to show the capabilities of this objective, which is normally made for use in ultraviolet and is corrected for use with a fused quartz cover, a diatom, pleurosigma angulatum was mounted on a quartz slide and covered by a quartz cover glass by John Butterfield and two photographs were made, one with wave-length 5460A, and the other in the region 2537 to 2650A. (Figure 8.) The picture taken in ultraviolet shows much better detail than the one taken in green. The diatom shows a hexagonal structure undoubtedly because the diffraction rings from the other direction of the markings are included in the aperture, since the diffraction beams for shorter wave-lengths are crowded closer to the direct pencil than are those of long wave-lengths. The illuminating aperture was 0.19 N.A., set sufficiently oblique to just clear the occluded aperture, which is 0.216 N.A.

PHASE CONTRAST MICROSCOPY

In addition to resolution of fine detail and the disclosure of absorption differences within biological material, through all parts of the spectrum, considerable progress has been made in the matter of rendering visible the optical path differences which exist in microscopic objects. This method of microscopy is known as phase-difference, or phase-contrast microscopy, and has been reported on by Zernike,[13] its inventor, and many others. A complete bibliography of phase difference

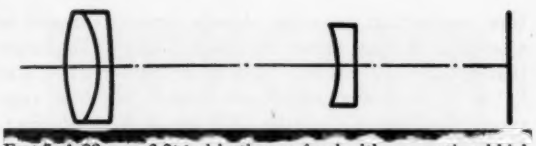
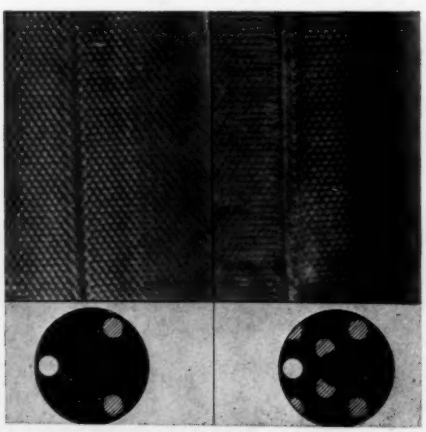


FIG. 7. A 28 mm, 3.2× objective parfocal with conventional high power microscope objectives.

microscopy has been brought up to date by American Optical Company.[14] This form of microscopy depends upon altering in phase and/or amplitude (i.e., path length and/or intensity), the pencil of undeviated light passing through the object and the objective, such that when the diffracted beams of light join with the direct beam, desired image contrast exists.

A synthetic test specimen for phase contrast microscopy has been executed by the use of evaporated magnesium fluoride film. A glass slide was coated with a thin film of magnesium fluoride having an optical path length of one-quarter wave-length. Wherever the film has been removed by scratching, an optical path difference of one-quarter wave-length exists. Since the thin film is quite transparent, one should not be able to see it. A series of pictures were taken of such a film with a 1.8 mm, 1.25 N.A. objective. The series was taken in brightfield illumination (Fig. 9a), bright phase contrast (Fig. 9b), dark phase contrast (Fig. 9c), and darkfield illumination (Fig. 9d). The picture with regular brightfield illumination lacks contrast between the scratch and the background. The bright phase picture shows the area within the scratch darker than the area around it, and the dark phase picture shows the area within the scratch lighter than the area around it. Dark (or positive) con-



(a) (b)

FIG. 8. Photomicrographs of pleurosigma angulatum and the appearance of the back aperture of the objective. The objective was the Polaroid-Grey 2.8 mm, 0.72 N.A. reflecting objective. (a) Photograph made in visual green, (b) photograph made in ultraviolet.

[6] L. V. Foster and E. M. Thiel, J. Opt. Soc. Am. 38, 689 (1948).
[7] B. K. Johnson, J. Sci. Inst. and Phy. Ind., 26, 193 (1949).
[8] E. M. Brumberg, Bull. Acad. Sci. U.R.S.S. 6, 32 (1942).
[9] C. R. Burch, Proc. Phys. Soc. (London) 59, 41 (1947).
[10] D. S. Grey and P. H. Lee, J. Opt. Soc. Am. 39, 719 (1949).
[11] D. S. Grey, J. Opt. Soc. Am. 39, 723 (1949).
[12] L. V. Foster, Anal. Chem. 21, 432 (1949).
[13] F. Zernike, Physica 9, 686, 974 (1942).
[14] Chart Compiled by American Optical Company.

trast means that retarding objects appear as denser or absorbing objects, hence, the MgF film will be darker than areas where there is no film in dark contrast and lighter than these same areas in bright (negative) contrast. The darkfield picture only shows an edge effect.

EYEPIECE

The compound microscope would not exist without the eyepiece, which is literally a magnifier to view the real image formed by the objective. The two basic forms of microscope eyepieces are the Huygenian, which is achromatic for focal lengths, and the compensating, which is overcorrected for focal lengths to compensate for the undercorrected chromatic difference of magnification existing in most microscope objectives. The objectives differ among themselves in this respect. Figure 10 shows a graph of the chromatic difference of magnification expressed in terms of the ratio of the focal lengths of spectrum lines *F* and *C*. This error is only evident toward the edge of the field where images will appear fringed with color in unbalanced systems. In visual work without filters and in color photomicrography, it is important to use an eyepiece and objective in which the compensation for lateral color is well adjusted. All eyepieces corrected to overcome chromatic differences of magnification in the objective will have a field diaphragm fringed with color. This is not a defect, but is indicative that the eyepiece is a compensating type.

Attempts to influence the curvature of field of the microscope have been made by the various microscope manufacturers in the form of special eyepieces. For several years Leitz has made the Periplan eyepiece, and Bausch & Lomb has made the Hyperplane eyepiece. Both eyepieces are Huygenian types designed similar to the compensating eyepiece and do introduce some overcorrection to field curvature. More effective in influencing field curvature is the Zeiss Homal eyepiece and the Bausch & Lomb Ampliplan, which are strong negative lenses used only in projection or photomicrography.

FIG. 9. Photomicrographs of tear in magnesium fluoride film on glass made with a 1.8 mm, 1.25 N.A. objective. (a) Regular bright-field illumination, (b) bright phase contrast, and (c) dark phase contrast, and (d) darkfield illumination.

SUBSTAGE CONDENSER

The microscope had not reached such a state of refinement when the first compound instrument was made by Galileo in 1610, that it required a substage condenser. It was not until 1691 when Bonanni, an Italian, described in his book Micrographia Curiosa, a compound microscope with a lamp and a substage condenser. It is quite evident now that illuminants and means to concentrate light under controlled angular apertures must be provided for the modern microscope. The purpose of the substage condenser is to direct beams of light through the object in such a way as to exhaust the capabilities of the objective. The object which must be illuminated is often mounted on the surface of a glass slide whose thickness may vary from 1.0 to 2.0 mm. Thus, the condenser must have its focal point quite a distance in front of its last surface. When the condenser is required to deliver a wide angular aperture of light to the object, it means that the focal length must be appreciably long in order that its working distance will be sufficient to work through this amount of glass. This requirement causes the lenses to be large in diameter and the whole condenser to become thick. A substage condenser which fills these nominal conditions is the well-known Abbe condenser made with either two or three lenses in various sizes, and focal lengths ranging in N.A. from 0.70 to 1.40. These condensers all have large amounts of chromatic and spherical aberration.

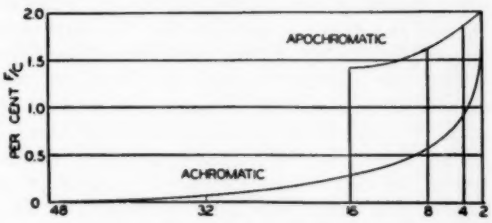


FIG. 10. Chromatic difference of magnification of achromatic and apochromatic objectives.

If the microscope is to be used for visual studies of highly stained thick sections as the average medical student uses it, the Abbe condenser of 1.0 N.A. is adequate. The illuminator for this kind of work does not need to be more elaborate than the surface of an inside frosted Mazda lamp of about 40 watts, placed about 12 inches in front of the microscope. When conditions require a critical balance of field illumination, control of the angle of the illuminating cone, with decentered illuminating pencil, and, if selected wave-lengths are employed,

nothing but a well-corrected condenser will suffice. Such a condenser should be corrected for chromatic aberration. Since the condenser generally images the illuminant in the object field, it is desirable that the introduction of color filters causes no change in the focal setting of the condenser. The condenser should be sufficiently well corrected for spherical aberration to permit decentration of the substage iris without causing the image of the light source to sweep across the field. Condensers which completely fill this requirement do not exist. Approaches to them have been made in the way of aplanatic and achromatic condensers. The former is full of chromatic aberration, but is to be preferred over an achromatic condenser not corrected for spherical aberration. Microscope objectives would make excellent condensers if the objects were mounted on cover glasses. If the regular oil immersion type of microscope objective were made 5 times its regular size, it would just work through a 1.5 mm object slide when the lenses would be about 1 inch in diameter and the total thickness would be 1.5 inches. Condensers like this have been made, but they are very heavy to mount and are much more expensive than more simple forms.

LAMPS

Since the microscope is not often used with daylight as an illuminant, the light source has become quite important to it, especially when used for projection or in photography. For use in photomicrography, the Ediswan Pointolite lamp was considered ideal. This lamp was originally made in England but, because of its popularity, was marketed here by General Electric Company as a Tungsarc lamp. It did not last long because of two reasons. It operated only on d.c. and the ball of tungsten oxidized rather rapidly, making an irregular source of light. The ribbon form of tungsten filament was gradually perfected, and a lamp of this type operating on 6 v at 18A on either d.c. or a.c., has replaced all the Pointolite lamps.

Next came the tungsten arc lamp, known popularly as the sun lamp, since it was a modification of the General Electric Type S-1. This lamp was very popular when it first came out but, because of the high socket temperature, and the variable life of the lamp, and the fragility of the bipost base, was dropped from the line only a few years after it was developed. Many improvements were made in the carbons for use in the d.c. arc lamp, and the lamp itself has been made fully automatic by the principal of advancing or separating the electrodes in accordance with the drop in potential across them. The Western Union zirconium arc is a rather new development in light source suitable for photomicrography and some microprojection. It promises to be extremely useful in microscopy. The Western Union Laboratories have developed zirconium electrodes, which may be burned in air, and which can be operated for about 1 hour without need for changing their position. This lamp

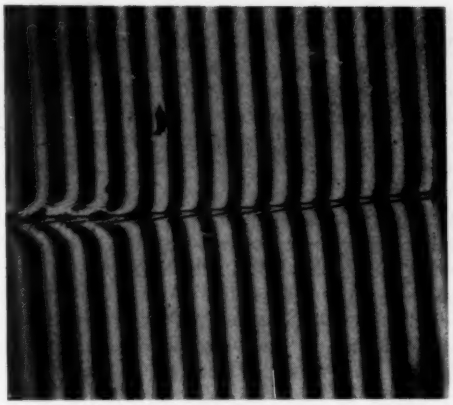


FIG. 11. Photomicrograph of diamond scratch on glass. By following interference bands across the scratch, an estimate of its depth can be made.

was recently described at the Society of Motion Picture Engineers Meeting in Los Angeles.

Small sources have not been so numerous. The small Mazda source, which was used in the early development of lamps, was the automobile dash type or, in some cases, the *V* type headlight. Neither were good and, in cooperation with Mr. Carlson of General Electric Company, a small lamp of 25 candle power was made by winding a tungsten wire on a mandrel about 2 mm in diameter. This gave a source about 2 mm square. It was first made in a bayonet base but has since been made on a prefocus base.

SPECIAL MICROSCOPES

All microscopes use objectives and eyepieces, but not all use substage condensers. The microscope used to examine opaque objects, such as metals and ores, uses only an objective and eyepiece, but between the two a vertical illuminator is placed to reflect light through the objective, which in this case functions also as the condenser. The object is then said to be illuminated with vertical incident light. Many improvements in this type microscope have been made over the last thirty years. The early metallurgical microscope made use of a thin plane glass plate inclined 45° to the objective axis for a vertical illuminator. This reflector directed about 10 percent of the initial light to the specimen and then transmitted about 90 percent of that returned by the specimen. The resulting intensity in the image could never be more than 9 percent of that starting at the source. Actually, it was not nearly as much, since the normal light losses at the objective were doubled, due to its functioning as both condenser and objective. The losses have been greatly reduced by the application of low reflection films to the surfaces of the objective lenses.[15] This has resulted in increasing the contrast in

[15] J. R. Benford, Trans. Am. Soc. Metals 36, 452 (1946).

details within objects of low reflectance. The low efficiency of the vertical illuminator has been changed by the application of a high index film, such as titanium dioxide to one side, and a low index film to the other side of the plane glass reflector, so that its over-all efficiency has been raised from 9 percent to 24 percent. An improved form of polarizing vertical illuminator with high efficiency was described before this society by the writer[16] in 1937. The efficiency of this illuminator is 31 percent. It has the advantage that it is a full aperture polarizer and analyzer at the same time, and thus provides the means for detecting anisotrapy in opaque materials. In 1945 Benford, Turner, and McLean[17] showed how measurements of bi-refringence could be performed by the addition of an elliptical vibration compensator to the equipment having the polarizing vertical illuminator.

Another form of microscope which may become popular after further development is the interference microscope. It has been described by Linnik[18] and a few of these instruments were made by Zeiss during the war. This microscope is actually an arrangement of two microscopes with their axes at angles and fitted with a system of vertical illumination. It utilizes two objectives of identical form and a beam splitter. In the object plane of one objective is a plane reflecting surface, and in the object plane of the other is the object to be tested. If the object under test is also plane and has the same reflectance as the comparative surface and the axial path length from object to image in the two beams is exactly alike, the field will appear bright. If the path lengths differ by multiples of a half-wave-length of light, the field will appear dark. Rarely will such a condition ever exist. Since either a bright field or a dark field carries no significance, means is provided for introducing a small wedge between the interfering beams, so that a series of interference lines cross the field. The object under test will have irregularities in it which may be elevations or depressions, and the heights or depths of these can be measured by following the interference bands across them. Figure 11 is a micro-interferogram of a diamond scratch on glass. The interference band contour indicates this scratch to be approximately one-half wavelength deep. Note that the surface contour raises slightly before dipping into the scratch. This effect has proceeded to the point of fracture as indicated at the left side of this slide.

[16] L. V. Foster, J. Opt. Soc. Am. **28**, 124 (1938).
[17] Turner, Benford, and McLean, Econ. Geol. **40**, 18 (1945).
[18] V. P. Linnik, Comptes rendus U.R.S.S. (No. 1) 21 (1933).

Symposium on Molecular Structure and Spectroscopy

The Symposium on Molecular Structure and Spectroscopy sponsored by the Department of Physics and Astronomy of the Ohio State University will be held June 12–17, 1950 at Columbus, Ohio. The following tentative program gives the subjects to be discussed and the names of the speakers who will begin the various sessions with a survey paper introducing the subject: June 12—**Microwave Spectroscopy,** Walter Gordy (Duke University); **The Theory of Infra-Red and Microwave Spectra,** Wave Shaffer (Ohio State University); June 13—**The Infra-Red Spectra of Molecules,** Shirleigh Silverman (Johns Hopkins University); **Ultraviolet Spectra of Molecules,** Richard C. Lord (Massachusetts Institute of Technology); June 14—**Spectroscopy of the Earth's Atmosphere,** Marcel Migeotte (Institut d'Astrophysique, Liege); June 15—**Raman Spectroscopy of Molecules,** Eugene Rosenbaum (Sun Oil Company); **Infra-Red Instrumentation,** John Strong (Johns Hopkins University); June 16—**Techniques in Infra-Red Spectroscopy,** Joseph R. Downing (E. I. du Pont de Nemours); **Chemical Analysis by Infra-Red Spectroscopy,** Norman Wright (Dow Chemical Company); June 17—**The Use of Infra-Red Spectroscopy for Biological Problems,** Gordon Sutherland (University of Michigan). Dean George R. Harrison (Massachusetts Institute of Technology) will be guest speaker at the dinner to be held Wednesday evening, June 14, 1950. Further information may be obtained from Professor Harald H. Nielsen, Mendenhall Laboratory of Physics, The Ohio State University, Columbus, Ohio.

A New Series of Microscope Objectives.* III. Ultraviolet Objectives of Intermediate Numerical Aperture†

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(Received December 19, 1949)

Paper II of this series demonstrates that spherical lens elements combined with spherical mirrors may provide well-corrected and achromatized ultraviolet microscope objectives. The present paper presents a further study of several types of catadioptric Schwarzschild systems which provide microscope objectives relatively easy to construct, convenient to use, and for which ultraviolet and visual range performance characteristics are not compromised. Several modifications with numerical apertures within the range 0.4 to 1.0 are presented.

INTRODUCTION

ATTEMPTS to solve the problem of correcting the chromatic aberrations of a microscope objective over the wave-length interval from about 220 to about 540 mμ led to the use of reflecting surfaces. Papers I and II of this series[1,2] have presented a preliminary study of catadioptric microscope objectives. As a consequence of the investigations reported in Papers I and II, it was decided to develop a series of catadioptric microscope objectives in which the reflecting surfaces consist of a Schwarzschild pair of mirrors (Fig. 1).

Three rules are observed throughout this investigation of ultraviolet objectives:

1. Only spherical surfaces are to be used.[3]
2. Only fused quartz and fluorite and possibly lithium fluoride are to be used for the refracting components.
3. Partially transmitting mirrors are not to be used; that is, an element of surface area may not function both as a mirror and as a transparent area.

One further rule was adopted after the completion of the studies reported in Papers I and II:

4. The objectives are to fit onto conventional microscope stands.

IMPROVEMENTS TO BE MADE ON THE SCHWARZSCHILD MIRRORS

The Schwarzschild mirrors with *spherical* surfaces and without auxiliary lens elements may be made sufficiently aberration-free for use as a microscope objective of numerical aperture about 0.5. The secondary mirror then obscures about 20 percent of the objective aperture area, and supports for this mirror add to the obscuration. Sacrifice of numerical aperture permits a small reduction of obscuring ratio.

Attempts to remove these limitations by modifying the Schwarzschild mirrors suggested the following desirable improvements listed in approximate order of importance:

1. To increase the numerical aperture,
2. to decrease the obscuring ratio,
3. to seal the system against dust on the mirrors,
4. to eliminate opaque supporting structure for the convex mirror, and to simplify the mechanical means by which this element is incorporated into the optical system,
5. to increase the centering and spacing tolerances of the mirrors with respect to each other,
6. to provide for correcting chromatic aberration introduced by the cover slip and eyepiece, if any.[4]

A "DRY" OBJECTIVE OF NUMERICAL APERTURE 0.7—OPTICAL DESIGN

A catadioptric Schwarzschild objective of numerical aperture 1.1 is described in Paper II of this series.[2] Although the computed performance is adequate, that objective is difficult to construct and is too large for any ordinary microscope stand. Rather than to improve upon this design immediately, it was decided that objectives of intermediate numerical apertures should be thoroughly investigated. The evolution of design types, the elimination of construction difficulties, and the evaluation of actual performance should be expected to proceed more easily if the numerical aperture is not so large that its attainment creates a major problem.

The first of these objectives was computed for a numerical aperture of about 0.7. There were two reasons for this choice. An objective of numerical aperture 0.7 and small obscuring ratio would represent a worth-while improvement over the performance attainable with the spherical and purely reflecting Schwarzschild type of objective. Also, an ultraviolet objective of numerical aperture 0.7 should have resolving power roughly equivalent to the best attainable in visible light.

After preliminary study, the general configuration shown in Fig. 2, was adopted. In this arrangement of components, the spherical aberration of the convex mirror is appreciably smaller in absolute value than is the spherical aberration of the concave mirror. It is this distinction between the objective of Fig. 2 and the objective described in Paper II of this series, for which the mirrors are nearly compensating in spherical aberration,

* This paper was presented in abbreviated form at the New York meeting of the Optical Society of America (March, 1949).
† The work reported in this paper was supported by the ONR.
[1] D. S. Grey and P. H. Lee, J. Opt. Soc. Am. 39, 719 (1949).
[2] D. S. Grey, J. Opt. Soc. Am. 39, 723 (1949).
[3] The Schwarzschild objectives reported by Burch are based on aspheric mirrors. C. R. Burch, Proc. Phys. Soc. London 59, 41 (1947).
[4] The objectives used by Brumberg provide a weak lens between the convex mirror and the long conjugate focus. The only apparent function of this lens is to correct chromatic aberration introduced by the cover slip. E. M. Brumberg, Nature (London) 152, 357 (1943).

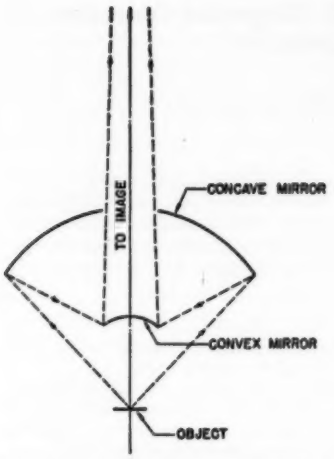

FIG. 1. The Schwarzschild mirrors illustrated above are the starting point for the design of a series of objectives. Lens elements are added to this basic pair of mirrors to increase the numerical aperture at which aberrations are corrected, to decrease the obscuring ratio, to seal the system against dust, and to provide a non-obscuring support for the convex mirror.

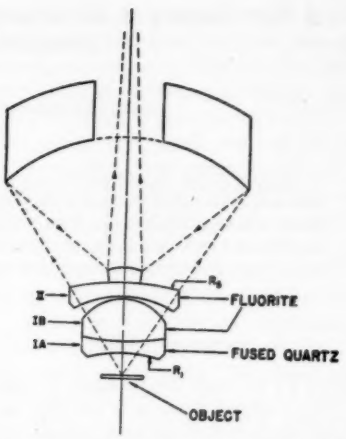

FIG. 2. Lens components have been added to the Schwarzschild mirrors to provide an objective of numerical aperture 0.72, corrected for use in the wave-length region from 220 mμ through the visible spectrum. Nine percent of the objective aperture area is obscured by the convex mirror.

which permits a small obscuring ratio with an objective of reasonable size.

As stated in Paper II of this series, if the net third-order spherical aberration of the mirrors is an undercorrection, the refractive components must provide overcorrected third-order and undercorrected higher order spherical aberration. This state of correction may be realized if there is a refracting surface of large spherical overcorrection (R_2 in Fig. 2), and a refracting surface of large spherical undercorrection (R_1 in Fig. 2), with the latter surface the nearer to the short conjugate focus. The required size of the respective third-order coefficients depends, among other things, on the separation between the surfaces and the net spherical aberration of the mirrors. Proper choice of these last two variables permits one to attain the desired residual third-order coma.

The objective may be corrected as an achromat (in the generally accepted usage of the term for microscope objectives) if the cemented component, elements *IA* and *IB*, is replaced by a simple lens of the same medium as lens *II* (either fused quartz or fluorite). In this case the variation of spherical aberration with wave-length may be corrected according to third-order theory, but I was unable to provide a residual third-order spherochromatic aberration of the correct algebraic sign to compensate a rather large fifth-order spherochromatic aberration. Also, this simplified configuration gives a residual secondary spectrum of anomalous sign.‡ Accordingly, lens element *IA* was introduced to correct these defects.

‡ Almost all achromatic lens combinations have a secondary spectrum such that for a positive lens the extremes of a spectral interval focus further from the lens than does the center of the spectral interval. Anomalous secondary spectrum is secondary spectrum opposite in algebraic sign to the usual type.

This negative lens of fused quartz may be given just sufficient power to compensate the anomalous secondary spectrum. The fluorite-fused quartz interface provided the desired variation of spherical aberration with wavelength. The most satisfactory over-all state of correction was achieved when the anomalous secondary spectrum was slightly overcompensated. The final state of correction is such that this objective is apochromatic over the interval 220 to 600 mμ; that is, throughout that interval aberrations and obscuration of aperture do not cause a loss of more than 22 percent in the percentage of transmitted energy which falls within the central disk of the diffraction pattern. The working distance of this objective is sufficiently great to permit insertion of a hemisphere in contact with the cover slip. This could give a numerical aperture of at least 1.1, hence the reduction in size relative to the objective described in Paper II of this series may be realized with no loss of performance.

A "DRY" OBJECTIVE OF NUMERICAL APERTURE 0.7, CONSTRUCTION AND EXPERIMENTAL RESULTS

The Bausch and Lomb Optical Company designed a mounting and executed experimental models of the objective. An indispensable feature of the mounting is that the centering and spacing of the mirrors may be adjusted empirically. The centering adjustment is lateral motion of lens element *II* and consequently of the convex mirror, the spacing adjustment is axial motion of the concave mirror. The criterion of proper adjustment is the visual appearance of out-of-focus-diffraction rings around the image of a pinhole target.

Much was learned in the course of executing the first models and in preliminary use of the objective:

(1) A plano component between the convex mirror and the long conjugate focus was added to seal against dust on the mirrors.

Optically, this element is a parasite, but it can be put to good use as a design parameter if its surfaces are curved. Subsequently this element has been given a slight curvature on one face, so that the longitudinal chromatic aberration of a Huygens eyepiece is compensated.

(2) Centering of the convex mirror on the lens element which supports it is not an easy operation.

(3) Only 25 percent of the total power is contributed by the refractive components. Consequently, the mirrors require critical alignment for numerical aperture 0.70.

Aside from these difficulties the objective performs satisfactorily.

AN IMMERSION OBJECTIVE OF NUMERICAL APERTURE 0.90

Numerical aperture 0.70 is rather large for critical use of a dry objective in ultraviolet, because the cover slip tolerance is extremely small. The tolerance on permissible variation of the thickness τ from the object to the top of the cover slip is plotted as a function of the numerical aperture of the objective in Fig. 3. This graph was computed on the assumption that error in the thickness τ may introduce an amount of spherical aberration not greater than one-half of the Rayleigh tolerance for wave-length 250 mμ.

Similarly, the tolerance on variation of index and dispersion of an immersion liquid from that specified is more strict for ultraviolet systems than for visual systems. Moreover, many standard immersion oils fluoresce in ultraviolet or have high absorption in ultraviolet. Water is a suitable immersion liquid for numerical

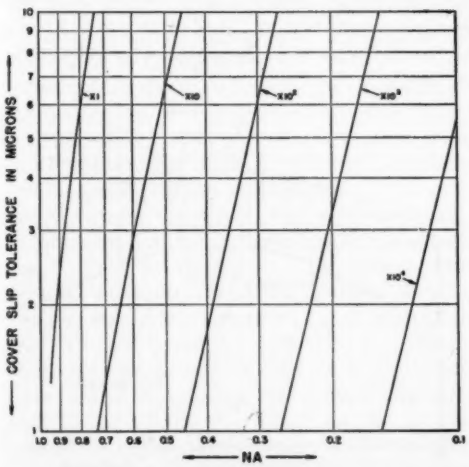

FIG. 3. Variation in cover slip thickness from the thickness assumed in lens computation introduces spherical aberration. The *total range* of permissible variation in the distance from the object plane to the top of the cover slip is plotted above as a function of numerical aperture of the objective. The computation is based on permitting the thickness variation to introduce an amount of spherical aberration equal to ¼ wave-length, or half the Rayleigh tolerance, at wave-length 250 mμ. The tolerance is proportional to wave-length. Computations are for cover slip index 1.5. The factors, $X1 \cdots X10^4$, associated with the curves indicate the factor by which the ordinate is to be multiplied to yield the tolerance for the corresponding abscissa, or NA, value.

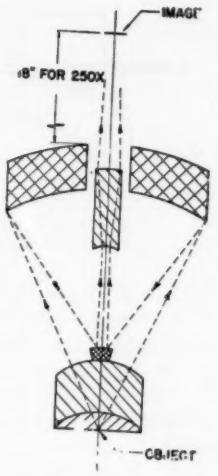

FIG. 4. This objective, computed for water immersion, can be well corrected at numerical apertures up to 0.90. Variation of magnification with wave-length may be eliminated, so that no eyepiece is necessary.

aperture less than unity; for higher numerical apertures it is well to match the index of the immersion liquid more closely to that of the adjacent refracting surfaces.[5] The objectives described in this paper are corrected for water immersion.

The objective of Fig. 2 was computed as a dry objective in accordance with the general practice for objectives of that numerical aperture. Many difficulties of constructing and using the objective, and virtually all the design problems are directly or indirectly a result of avoiding immersion. The immersion objective shown in Fig. 4 may be adequately corrected for numerical apertures up to about 0.90 (or numerical aperture 0.95 in very short focal lengths).

A distinct advantage of this objective (Fig. 4) is that chromatic variation of magnification may be corrected by the lens situated between the convex mirror and the long conjugate focus; there is thus no need for an eyepiece. (The term eyepiece is here used to describe any type of optical system which relays the primary image, formed by the objective, into a magnified image which is to be photographed, viewed with fluorescent screen, etc.). For the objective illustrated, a magnification of 300 diameters may be obtained with a distance from object to image of 24 inches. This magnification is sufficient for use with a fine-grain photographic process. When this objective is used without eyepiece, there are only three air-exposed refracting surfaces between object and image; thus it should be almost entirely free from scattered light.

ELIMINATION OF THE SEPARATE CONVEX MIRROR

There is a final general problem which should be investigated before these objectives are adapted to quantity production. It is desirable that the convex mirror and the lens surface which supports it have the

[5] For a discussion of glycerin-sugar-water mixtures for immersion liquids, see L. C. Martin and B. K. Johnson, J. Sci. Inst. 5, 380 (1928).

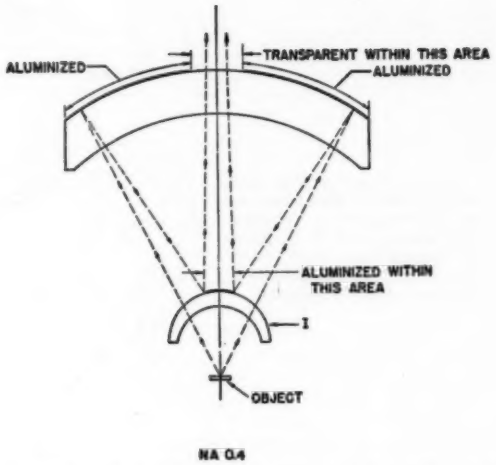

FIG. 5. A method of eliminating the construction and mounting of a separate convex mirror element is illustrated above. The convex mirror is continuous with a lens surface, and is merely an aluminized spot thereon. The concave mirror is back surfaced; the front and refracting surface of this lens element provides a correction for the chromatic aberration of the lens element adjacent to the object.

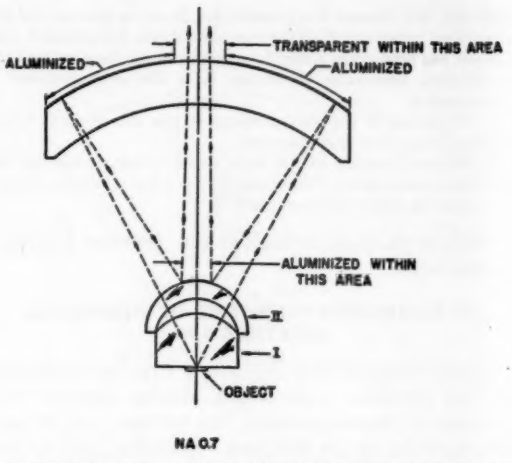

FIG. 6. Objectives similar to that of Fig. 5 may be given additional refractive components to provide correction at larger numerical apertures.

same curvature and the same location. The convex mirror then becomes merely an aluminized spot at the center of a lens. The small convex mirror is no longer an additional element to construct and, even more important, the operation of mounting the convex mirror is eliminated.

The three catadioptric Schwarzschild objectives illustrated in Figs. 2 and 4 of this paper and in Fig. 1 of Paper II have this feature in common: The lens surface on which the convex mirror is mounted is much less steeply curved than is the convex mirror itself. If this lens surface is strongly curved, a large amount of chromatic aberration is introduced. It is difficult to remove this chromatic aberration by placing achromatizing elements between this lens surface and the short conjugate focus because the fused quartz-fluorite combination does not provide a large dispersion difference.¶ Moreover, the methods heretofore described for controlling spherical aberration and coma have used a free choice of curvature for the lens surface nearest the convex mirror. The convex mirror arrangement proposed creates, therefore, a new problem of chromatic aberration, and eliminates the parameter discussed above for control of spherical aberration. Methods by which control over chromatic aberrations and spherical aberration may be regained in the presence of the proposed convex mirror treatment are perhaps best understood if preceded by a few general remarks on chromatic aberration.

When an expression for the longitudinal chromatic aberration of a lens system is derived, the product $h_K(\Delta\theta)_K$ appears as a term of major importance. The quantity h_K is the incident height of a ray at the Kth surface and $(\Delta\theta)_K$ is the amount of refraction at the Kth surface. The term $h_K(\Delta\theta)_K$ indicates the difficulty which arises in compensating the chromatic aberration introduced by a strongly refracting surface at which h is large. But if the chromatic aberration is compensated at a surface with a yet larger h value, the difficulty is resolved.

If the concave mirror is a backcoated mirror, the front (refracting) surface of this element may provide chromatic overcorrection. This surface will have an h value much larger than does any other refracting sur-

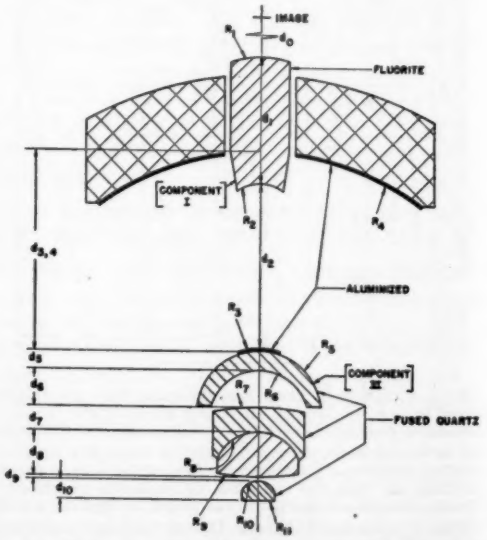

FIG. 7. Another method of "eliminating" the convex mirror element is shown above. Numerical aperture 1.0, obscured area 9 percent. Residual aberrations shown in Figs. 8 to 10.

¶ For fused quartz $n_{270}(n_{220}-n_{270})^{-1}=9.11$; for fluorite $n_{270}(n_{220}-n_{270})^{-1}=12.57$.

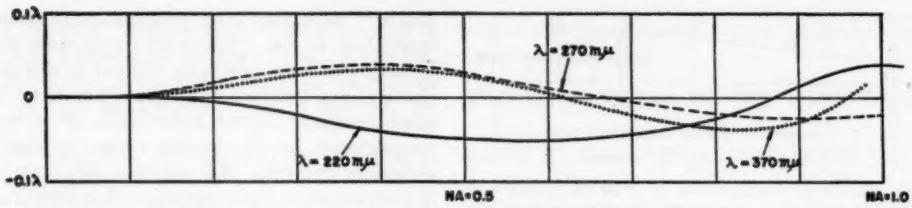

FIG. 8. Spherical aberration in terms of phase retardation for Fig. 7 type objective. These curves were computed for a common focal setting at the three wave-lengths, and thus *include* the effect of the shift of the plane of best focus.

face, and hence may easily compensate chromatic aberration of the objective as a whole. One group of objectives which have the convex mirror continuous with a lens surface is based on this arrangement.

Another device used to eliminate the separate convex mirror is to introduce a diverging lens between the convex mirror and the long conjugate focus. The diverging power of this lens substitutes in part for the diverging power of the convex mirror. The curvature of the mirror surface is thus decreased, and it may more easily be used both as a mirror and as a lens surface.

OBJECTIVES BASED ON A BACK-COATED CONCAVE MIRROR

Figures 5 and 6 illustrate two objectives which use a backcoated concave mirror to correct chromatic aberration.

The dry objective (Fig. 5) could be used at numerical apertures of about 0.5 over a restricted band width. There are residuals of spherochromatic aberration and secondary spectrum which restrict the numerical aperture to about 0.4 if aberrations over the interval 220 to 540 mμ are to be negligible.

The secondary spectrum is anomalous and arises from surface separation. Secondary spectrum may be essentially eliminated if the lens-mirror element *I* is of fluorite.|| Spherochromatic aberration is also substantially reduced by this modification.

The immersion objective (Fig. 6) is corrected for secondary spectrum but has residual spherochromatic aberration. This aberration may be reduced, without introducing secondary spectrum, if lens *II* is fused quartz and lens *I* is a cemented fused quartz-fluorite doublet. The performance of both these objectives may be improved substantially if the central portion of the concave mirror is replaced by a freely chosen lens element.

Exhaustive exploration of these design possibilities was postponed until one model was constructed and tested. Several features of these designs might make the execution difficult. First of all it should be noted that there is a long path length through fused quartz, and that thick, large diameter pieces of this material are required. Probably the finest fused quartz obtainable would be needed for the back-coated concave mirror. Suitable material is now available from the Heraeus Company, Hanau, Germany. Secondly, the lens-mirror elements are difficult to construct because the two surfaces of each lens-mirror element are nearly concentric. This would not be a major problem were it not that the cost and restricted supply of the best fused quartz places a premium on construction without reject elements. Finally, backsurfacing the concave mirror divides the figure tolerance by the index of refraction of the optical medium.

A SECOND METHOD OF "ELIMINATING" THE CONVEX MIRROR

Figure 7 shows a second arrangement of components wherein the convex mirror is an aluminized spot on the center of a convex lens surface. Lens element *I* acts as a diverging lens which reduces slightly the curvature required of the convex mirror, $R_{3,5}$. The chromatic aberration introduced by $R_{3,5}$ as a refracting surface is materially reduced and may more easily be corrected by surfaces nearer to the short conjugate focus. Indirectly, lens element *I* further lessens the difficulty of eliminating chromatic aberrations, by providing additional variables for solving third-order aberrations. The radii R_8 to R_{11} may be more freely chosen when element *I* is utilized as a design parameter.

The computation of this objective presented two problems in eliminating aberrations which did not have obvious solutions and which could not be treated by methods already described in this series of papers.

The first problem was to discover a general arrangement which permits solution of third-order aberrations at two wave-lengths and which permits achromatization and elimination of secondary spectrum. This investigation was performed entirely by Gaussian optics and algebraic third-order theory, and led to the general form illustrated in Fig. 7.

The objective of Fig. 7 has one great simplification from the designer's point of view over that shown in Fig. 2—there is nearly independent control of spherochromatic aberration and of secondary spectrum. Various parameters affect these aberrations. The final controls employed were the paraxial incident heights at

|| Dr. James G. Baker suggested that (large) lenses of synthetic fluorite may give an appreciable amount of light scatter at the lens surfaces. Dr. Baker's experience was not directly applicable, but indicated the desirability of testing the scatter of ultraviolet radiation at aluminum-on-fluorite mirrors. Test samples of flat aluminum-on-fluorite mirrors were prepared by the Bausch and Lomb Optical Company and appeared to be free of this possible defect.

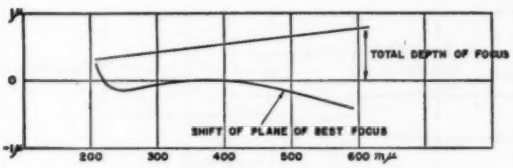

FIG. 9. The shift of the plane of best focus for the objective of Fig. 7, and the total depth of focus at numerical aperture 1. The shift of the plane of best focus includes variation of spherical aberration with wave-length. The allowance of chromatic overcorrection to compensate an unachromatized eyepiece has been subtracted from these residuals.

surfaces 9 and 8. When the paraxial incident height at surface 9 is increased,** spherical aberration at short wave-lengths becomes more overcorrected relative to spherical aberration at long wave-lengths. When the paraxial incident height at surface 8 is increased, secondary spectrum becomes more anomalous in character. These controls are, of course, not completely independent, but are sufficiently independent that there is no great difficulty in obtaining the desired residual aberrations.

The second problem was to eliminate a variation of fifth-order coma with wave-length. The road to discovering a control over this aberration was opened when it was noticed that one of the earlier trial designs was free of this defect, but that *all* later trial designs had a fifth-order difference O.S.C. (offense against the sine condition) of about 0.009 between 220 and 370 mμ. In all of these systems the angle of incidence at the concave surface of lens component *III* was large; and showed a large difference between wave-lengths 220 and 370 mμ. Moreover, the system which was free of *chromatic* variation of O.S.C. had the least large angle of incidence at this surface and the smallest variation of this angle with wave-length. Both of these parameters (size of angle of incidence and variation of this angle with wave-length) were tried, both separately and together, as a means of regaining control over the aberration, but with no success. The O.S.C. variation persisted at about 0.009 over relatively large variations of these parameters, and even through major rearrangements of components. It was finally found that the chromatic variation of O.S.C. could be correlated to the amount by which third-order spherical aberration is undercorrected (considering light traveling from the long to the short conjugate focus) prior to incidence on the concave surface of component *III*. This correlation may indicate either a direct cause and effect relation, or may result from a constrained relation between the parameter and the true source of control.

Other higher order aberrations were easily handled. Higher order spherical aberration could be correlated to the third-order spherical aberration contributed by surface 9,** and higher order monochromatic coma could be correlated to the third-order coma from surface 10. Coma control was not fully utilized in this design; mechanical construction is simpler when a small residual monochromatic higher order coma is permitted and compensated by third-order coma. A small residual secondary spectrum of anomalous sign was introduced to compensate a slight variation of spherical aberration with secondary spectrum.

The magnification is 2.0 percent greater at $\lambda = 220$ mμ than at $\lambda = 370$ mμ. An eyepiece may easily compensate this variation. Longitudinal chromatic aberration is overcorrected by 3 mm (between 220 and 370 mμ) at the *long* conjugate focus. This residual is to permit an eyepiece uncorrected for longitudinal chromatic aberration.

The residual aberrations of the objective are given in Figs. 8 to 10.

EFFECTS OF SHORT OBJECT DISTANCE AT THE CONVEX MIRROR

The objective described in the previous section uses a negative lens between the convex mirror and the long conjugate focus, and certain advantages thereby provided are described. One effect in particular will be treated here in detail, for it has a direct bearing on the general consideration of Schwarzschild Systems.

Let us return for the moment to the pure reflecting system, and discuss it as for *light traveling from the long conjugate focus to the short conjugate focus.*

One of the primary defects of this system and the only one which cannot be compensated by trivial modifications when the numerical aperture is of the order of 0.5 or less is obscuration of aperture. This has a certain minimum value dependent on the tolerance for spherical aberration and coma.

Now let the object (under the italicized convention above) as seen at the convex mirror be real and bring the object progressively closer to the convex mirror. During this process, change the curvature of the convex mirror so that the *image* distance after reflection at the convex mirror is constant. As the image distance decreases, the absolute value of the third-order spherical aberration from the convex mirror *increases* until a very small image distance is reached. This change in aberration of the *convex* mirror increases that spacing between the concave and convex mirrors which provides zero net third-order spherical aberration and coma, and gives a consequent *reduction in obscuring ratio.*

The relation between obscuring ratio and magnification may be easily represented in equation form for the case that third-order coma and third-order spherical aberration are zero. Although this exact condition may not be desired in practice, an analysis of the case is indicative of the effect of magnification on obscuration.

If third-order spherical aberration and coma are both

** That is, when the paraxial incident height is increased relative to paraxial incident height at surfaces 7 to 1. Computations were performed for light traversing the objective from the long conjugate focus to the short conjugate focus.

** Surface 9 thus provides two very important controls which are distinctly different both as to parameter and aberration affected.

eliminated, the mirror surfaces have a common center of curvature. Let d denote the distance from the common center to the object point, and R_1 and R_2 the radii of curvature of the convex and concave mirrors, respectively.

Then the condition for removal of third-order spherical aberration is:

$$\frac{R_1}{R_2}=\frac{3}{2}-\frac{R_1}{d}-\left(\frac{5}{4}-\frac{R_1}{d}\right)^{\frac{1}{2}}. \tag{1}$$

Third-order coma is eliminated by the concentricity of the mirrors.

The percent of aperture obscured is given by Q^{-2} where,

$$Q=\frac{3dR_2-R_1R_2-2R_1d}{R_2(d-R_1)}. \tag{2}$$

The magnification M from long conjugate focus to short conjugate focus is given by

$$M=\{1-(d/R_1)+2[(d/R_1)-1]^{\frac{1}{2}}\}^{-1}. \tag{3}$$

From these equations, Q may be expressed in terms of M.

$$Q=[2M+(5M^2-6M+5)^{\frac{1}{2}}][1-M]^{-1}. \tag{4}$$

From Eqs. (3) and (4) it is seen that as d becomes nearly equal to $2R_1$, the magnification approaches unity and the obscuring ratio approaches zero.

Now set $m=1/M$ so that m will represent the magnification of the objective used in the conventional sense, that is, with object at the short conjugate focus. Then obscured area may be plotted as a function of m (Fig. 11). From the appearance of this graph, one may expect that the following two general conclusions are valid:

(1) If a purely reflecting objective may be used at low initial magnification, the obscuration is thereby greatly reduced.

(2) A negative lens placed between the convex mirror and the long conjugate focus will reduce d and thereby reduce the degree of modification necessary to provide a low obscuring ratio.

REFRACTIVE POWER *VS.* CHROMATIC ABERRATION

It is stated above that we desire to modify the Schwarzschild mirrors so as to increase the centering and spacing tolerances of the mirrors. This may be accomplished by introducing lens components which contribute a large portion of the total power of the system. The mirrors themselves then work effectively at a smaller numerical aperture than does the objective as a whole. The individual aberration coefficients of the mirrors are thus reduced, and alignment tolerances are increased. In the objective described (Fig. 7), over half the power is derived from the lenses near the short conjugate focus. This creates an apparent paradox: Mirrors are used to provide the converging power of the system, because the secondary chromatic aberration of a refractive system is many times too great. After a system is devised based on a pair of mirrors, it is then found that the tolerances on locating the mirrors with respect to each other may be excessively small. To avoid this difficulty, refractive converging power is introduced into the system. How can a large amount of refractive power be introduced without causing a large amount of secondary spectrum to reappear?

There are two answers to this paradox. First, one may use an immersion system in which the lens nearest the object has center of curvature at the axial object point. This lens element multiplies the power of the system by the index of the refractive medium, and introduces no longitudinal chromatic aberration. The second answer is more easily understood if again light is considered to be traveling from the long conjugate focus to the short conjugate focus. Light traveling in this reverse direction is convergent after reflection at the concave mirror. If at a lens surface near the concave mirror the light is rendered slightly less convergent, chromatic overcorrection occurs. If at a surface more remote from the concave mirror this chromatic overcorrection is compensated using the same refractive medium as previously,

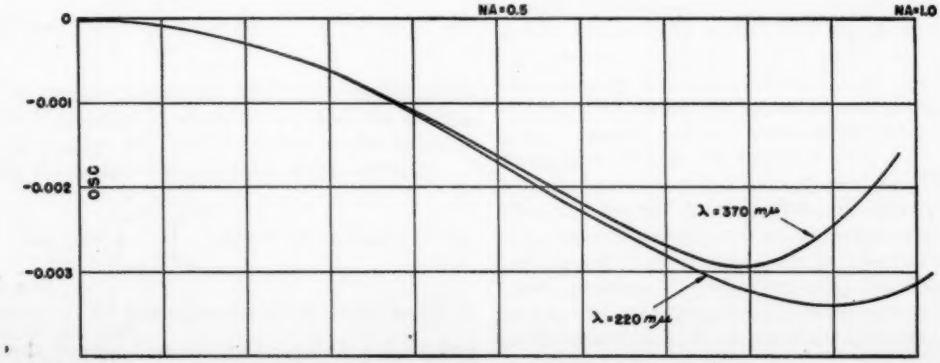

FIG. 10. Offense against the sine condition for the objective of Fig. 5. Negative offense against the sine condition means an increase in magnification.

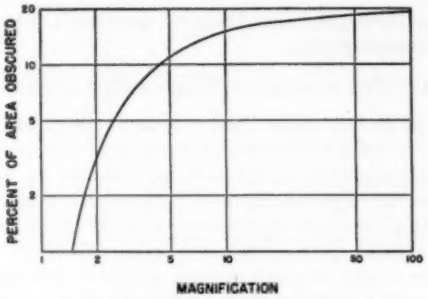

FIG. 11. If the Schwarzschild pair of spherical mirrors is used without auxiliary lens elements, the obscuring ratio decreases as the magnification for which the objective is computed is decreased. If third-order coma and spherical aberration are both zero, the obscured area varies with magnification as shown in this figure.

one finds that the convergence of the light has been increased by the two refracting surfaces. Moreover, the secondary spectrum from these refractive surfaces is anomalous in sign. When a large amount of refractive power is introduced, the secondary spectrum may be either anomalous or ordinary in character. Proper balance between obtaining chromatic correction by means of surface separations as above, and by the ordinary use of compensating "crown" (fluorite) and "flint" (fused quartz) components, can eliminate secondary spectrum. Residual longitudinal chromatic aberration is then more than adequately corrected for the range 220 mμ through the visible region.

ON OBTAINING THE ULTRAVIOLET FOCAL SETTING

The microscope objectives described in this series of papers are designed to provide a common focal setting for visible light and ultraviolet light, and to give an essentially aberration-free image at all these wavelengths.

No requirement against chromatic aberration, however strict it may be, will ensure that a microscope focused in visible light will be in exact focus for ultraviolet light. At a given numerical aperture, the visual depth of focus is greater than the ultraviolet depth of focus by a factor of about two. Furthermore, the appearance of a specimen is not the same in ultraviolet light as in visible light; the visually chosen vertical section of a specimen may not be *exactly* that desired for ultraviolet examination. For the most critical applications, it might be desirable to incorporate a focusing motion of the eyepiece.

ACKNOWLEDGMENT

The author wishes to thank Priscilla S. England for her valuable assistance in conducting the investigations reported in this paper.

An Extension of Toraldo's Theory of Phase Contrast I*

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The problem of phase contrast image formation with Köhler illumination is a special case of a more general problem which has been treated by Duffieux and his students: The Fraunhofer diffraction pattern due to the object is projected upon the phase pattern, and the diffraction pattern due to the latter constitutes the image. A semi-infinite plane covered with a uniform, transparent phase-shifting layer is to be regarded as a typical microscopic object. The variation in the appearance of the image as the light goes through different portions of the phase annulus is examined. The field is darkened on one side of the edge of the object and brightened up on the other side. If it is desired that the dark side shall be as dark as possible if a large portion of the annulus is filled with light, a quarter-wave phase shift must be introduced in the phase annulus. For light passing very close to the edge of the annulus, point-for-point image formation is no longer rigorously possible. As the annulus is made broader, the contrast becomes less pronounced.

THE problem of image formation in phase contrast is a special case of a more general problem treated by P. M. Duffieux[1] and his students: Collimated light traverses an aperture containing a non-uniform phase-shifting and absorbing layer (the object). A lens system (the microscope objective) projects the Fraunhofer diffraction pattern due to the object upon a second aperture also containing a non-uniform absorbing and phase-shifting layer (the phase pattern). To find the light intensity distribution in the diffraction pattern due to the latter aperture (the image).

Let x, y be Cartesian coordinates in the object plane, where the origin is on the axis of the microscope. Let α_1, β_1 be the direction cosines of the incident wave normal along the x- and y-axis, and let α_2, β_2 be the direction cosines of the diffracted wave normal. All linear dimensions are measured in units of one wave-length. The complex amplitude is introduced as a complex number such that its modulus represents the amplitude of the light wave and its argument represents the phase. Let $\mu=\alpha_2-\alpha_1$, $\nu=\beta_2-\beta_1$, and let $f(x, y)$ be a function such that its product by the complex amplitude of the light incident upon the object gives the complex amplitude of the light emerging from the object. The complex amplitude incident upon the phase plate is given by

$$g(\mu, \nu)=\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} f(x, y)e^{-2\pi i(\mu x+\nu y)}dxdy. \qquad (1)$$

Then the complex amplitude emerging from the phase plate is $P(\mu, \nu)g(\mu, \nu)$, where P is called the coating function. Finally, the light intensity in the image is given by the square of the modulus of the function

$$u(x, y)=\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} Pge^{+2\pi i(\mu x+\nu y)}d\mu d\nu. \qquad (2)$$

This "image" has been projected back into the object space according to the laws of geometrical optics.

Suppose Köhler illumination, and consider the light coming from one point of the light source (the light source is regarded as incoherent), then the contribution of each point of the source to $|u(x, y)|^2$ will have to be integrated over the utilized portion of the source.

Now suppose that, for any one value of x, f is a constant, so that it is a function of x only. Then g will vanish whenever $\nu\neq 0$. The diffraction pattern in the back aperture of the objective therefore falls on the line $\alpha_2=\alpha_1$, and the double integral in Eq. (2) reduces to a single integral along this line. Hence:

$$u=\int_{-\infty}^{+\infty} Pe^{+2\pi i\mu x}\int_{-\infty}^{+\infty} fe^{-2\pi i\mu x}dxd\mu, \qquad (2a)$$

where the integration with respect to x is to be performed first. If $P=1$ identically, u is the representation of f by a Fourier integral. Now α_1 and α_2 cannot be greater than 1 in absolute value, except for evanescent waves, which the objective cannot gather up. Therefore, in practice, the integration with respect to μ in Eq. (2a) is always extended over a finite domain. If $\alpha_1=0$, then, for the circular pupil of a coaxial optical system, the domain of this latter integration is symmetrical. Then, in the vicinity of a discontinuity of the function f, the graph of u will show certain peculiarities known as the Gibbs phenomenon. Hence, if f is constant except at a single discontinuity, which may be taken at $x=0$ without loss of generality, we have a typical microscopic object. For phase contrast, one is interested mainly in perfectly transparent objects, for which $|f|=1$.

These facts were first pointed out by G. Toraldo di Francia,[2] who calculated the light intensity in the image of a uniform, perfectly transparent, phase-shifting semi-infinite plane for the cases of bright field, dark field, and phase contrast. He supposes axial illumination, i.e., $\alpha_1=\beta_1=0$, and he supposes as a phase pattern, a circular patch concentric with the rim of the aperture. A number

* Presented at the Buffalo meeting of the Optical Society of America, October 1949.

[1] Duffieux, Tirouflet, Guenoche, and Lansraux, Ann. de physique 19, 355 (1944). Note the misprint on p. 369: Eq. (43) should not contain the factor of ½. Equation (41) is correct.

[2] G. Toraldo di Francia, Nuovo Cimento [9], 6, 30 (1949).

of other investigators[3-6] have developed the same theory from slightly different points of view. Their work for the most part contains the same conclusions by implication. Thus Bennett, Jupnik, Osterberg, and Richards[5] have shown that if the object is a combination phase-amplitude grating, the complex amplitude in the image is given by a Fourier series instead of the Fourier integral of Eq. (2a). But a Fourier series, if not summed to infinity, will also exhibit the Gibbs phenomenon.

The representation of the function f by the Fourier integral Eq. (2a) is not possible unless $f\to 0$ at great distances from the origin of x and y in such a manner that certain conditions of convergence are satisfied. Toraldo[3] avoids this difficulty by interchanging the order of the two integrations in Eq. (2a). If we are justified in assuming that any peculiarities in the behavior of $|u|^2$ are significantly intense only in the vicinity of the discontinuities of f which cause them, we may perform the integrations in (2a) more easily by setting $f=0$ for $|x|>a$, i.e., by bounding the illuminated portion of the field of view. If, in the resulting formula, $a\to\infty$, the resulting intensity distribution will then be due to only those specific object structures. However, there is described an example of diffraction where this assumption is not justified.

Consider now the object investigated by Toraldo. Let $f=e^{-i\varphi}$ for $-a<x<0$ and $f=e^{+i\varphi}$ for $0<x<+a$. In the back focal plane of the objective is the phase plate (Fig. 1), which bears the phase annulus. Within this annulus, $P=\tau e^{i\gamma}$; elsewhere, $P=1$ within the aperture of the objective. Without loss of generality, we may require $\tau\geqslant 0$. The geometrical image of the point of the light source under consideration is formed on the phase plate at $\alpha_1=\alpha_2$, $\beta_1=\beta_2$. We shall refer to this image as the pinpoint of light. The diffraction pattern about this point will fall along the line $\beta_2=\beta_1$. It crosses the edge of the aperture of the objective at $\alpha_2=\pm t$, and the edges of the phase annulus, at $\alpha_2=\pm q$ and $\alpha_2=\pm s$, where $0<q<\alpha_1<s<t$. Then $P=0$ for $|\alpha_2|>t$, $P=1$ for $t>|\alpha_2|>s$ and $|\alpha_2|<q$, and $P=\tau e^{i\gamma}$ for $s>|\alpha_2|>q$. These values must be substituted into Eq. (2). After the integrations have been carried out, we take the limit as $a\to\infty$. The resulting formula is:

$$\begin{aligned}u=&\tau\cos\gamma\cos\varphi+(1/\pi)\sin\varphi\{-\mathrm{Ci}[2\pi x(\alpha_1+t)]\\&+(1-\tau\cos\gamma)\mathrm{Ci}[2\pi x(\alpha_1+s)]\\&+(\tau\cos\gamma-1)\mathrm{Ci}[2\pi x(\alpha_1+q)]\\&+(1-\tau\cos\gamma)\mathrm{Ci}[2\pi x(\alpha_1-q)]\\&+(\tau\cos\gamma-1)\mathrm{Ci}[2\pi x(s-\alpha_1)]+\mathrm{Ci}[2\pi x(t-\alpha_1)]\\&-\tau\sin\gamma\mathrm{Si}[2\pi x(\alpha_1+s)]+\tau\sin\gamma\mathrm{Si}[2\pi x(\alpha_1+q)]\\&-\tau\sin\gamma\mathrm{Si}[2\pi x(\alpha_1-q)]\\&-\tau\sin\gamma\mathrm{Si}[2\pi x(s-\alpha_1)]\}+i\tau\sin\gamma\cos\varphi\\&+(i/\pi)\sin\varphi\{-\tau\sin\gamma\mathrm{Ci}[2\pi x(\alpha_1+s)]\\&+\tau\sin\gamma\mathrm{Ci}[2\pi x(\alpha_1+q)]\\&-\tau\sin\gamma\mathrm{Ci}[2\pi x(\alpha_1-q)]\\&+\tau\sin\gamma\mathrm{Ci}[2\pi x(s-\alpha_1)]+\mathrm{Si}[2\pi x(\alpha_1+t)]\\&-(1-\tau\cos\gamma)\mathrm{Si}[2\pi x(\alpha_1+s)]\\&-(\tau\cos\gamma-1)\mathrm{Si}[2\pi x(\alpha_1+q)]\\&-(1-\tau\cos\gamma)\mathrm{Si}[2\pi x(\alpha_1-q)]\\&+(\tau\cos\gamma-1)\mathrm{Si}[2\pi x(s-\alpha_1)]\\&+\mathrm{Si}[2\pi x(t-\alpha_1)]\},\end{aligned}\tag{3}$$

where

$$\mathrm{Si}(z)=\int_0^z(\sin\mu/\mu)d\mu\tag{4a}$$

$$\mathrm{Ci}(z)=-\int_z^\infty(\cos\mu/\mu)d\mu\tag{4b}$$

$$\lim_{z\to\infty}\mathrm{Si}(z)=\tfrac{1}{2}\pi\tag{5a}$$

$$\lim_{z\to\infty}\mathrm{Ci}(z)=0\tag{5b}$$

$$\lim_{z\to 0}[\mathrm{Ci}(Az)-\mathrm{Ci}(Bz)]=\ln|A/B|.\tag{5c}$$

If the annulus is narrow, the quantities α_1-q and $s-\alpha_1$ will be small compared to the quantities α_1+t, α_1+s, α_1+q, and $t-\alpha_1$. We may then classify the sine and cosine integrals in Eq. (3) as functions of small or large argument depending on which of these factors the argument contains. In this case, there will exist a range of values of $|x|$ such that the functions of large argument have already approached fairly close to their asymptotic values, while the functions of small argument still have approximately the same values as for $x=0$. With this approximation, we have from (3):

$$\begin{aligned}u\doteq&\tau\cos\gamma\cos\varphi+(1/\pi)\sin\varphi(1-\tau\cos\gamma)\\&\times\ln|(\alpha_1-q)/(s-\alpha_1)|+i\{\tau\sin\gamma\cos\varphi\\&+(1/\pi)\sin\varphi[-\tau\sin\gamma\ln|(\alpha_1-q)/(s-\alpha_1)|\pm\pi]\},\end{aligned}\tag{6}$$

where the upper sign is used for $x>0$, and the lower sign, for $x<0$. It will be seen that the two discontinuities in the pupil at $\alpha_2=-s$ and $\alpha_2=-q$ do not contribute to

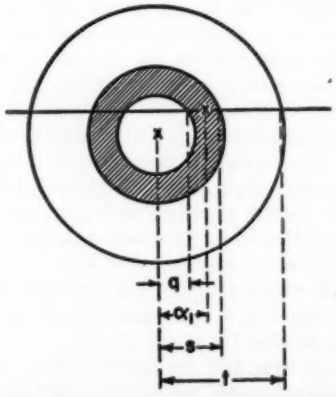

FIG. 1. Pupil of objective with phase annulus, showing the notation.

[3] M. Françon, Rev. optique 25, 257 (1946).
[4] J. Picht, Zeits. f. Instrumentenk 56, 363 (1936); 56, 481 (1936); 58, 1 (1938).
[5] Bennett, Jupnik, Osterberg, and Richards, Trans. Am. Microscop. Soc. 65, 99 (1946).
[6] E. G. Ramberg, J. App. Phys. 20, 441 (1949).

Eq. (6), so that this equation is valid for a phase strip or patch as well as for an annulus. The two ranges of values of x, for which this approximation is valid, are called the metastable regions, and the complex amplitude in these regions is denoted by u_m. The illumination in these regions is given by

$$|u_m|^2=\ln^2|(\alpha_1-q)/(s-\alpha_1)| \times(1/\pi^2)\sin^2\varphi(1+\tau^2-2\tau\cos\gamma) +\ln|(\alpha_1-q)/(s-\alpha_1)| \times(2\tau/\pi)\sin\varphi[\cos(\varphi\pm\gamma)-\tau\cos\varphi] +\tau^2\cos^2\varphi+\sin^2\varphi\pm 2\tau\sin\varphi\cos\varphi\sin\gamma. \quad (7)$$

The intensity in the metastable regions is a quadratic function of $\ln|(\alpha_1-q)/(s-\alpha_1)|$. Hence, for each metastable region and each value of β_1, there exists a value of α_1 such that $|u_m|^2$ is a minimum. This minimum is located at

$$\ln|(\alpha_1-q)/(s-\alpha_1)|=-\tau\pi[\cos(\varphi\pm\gamma) -\tau\cos\varphi]/\{\sin\varphi[1+\tau^2-2\tau\cos\gamma]\}. \quad (8)$$

Its value is

$$|u_m|^2_{\min}=[\tau\sin(\gamma\mp\varphi)\pm\sin\varphi]^2/(1+\tau^2-2\tau\cos\gamma). \quad (9)$$

If, in Eq. (9), the denominator does not vanish, $|u_m|^2_{\min}=0$ if

$$\tau=\mp\sin\varphi/\sin(\gamma\mp\varphi). \quad (10)$$

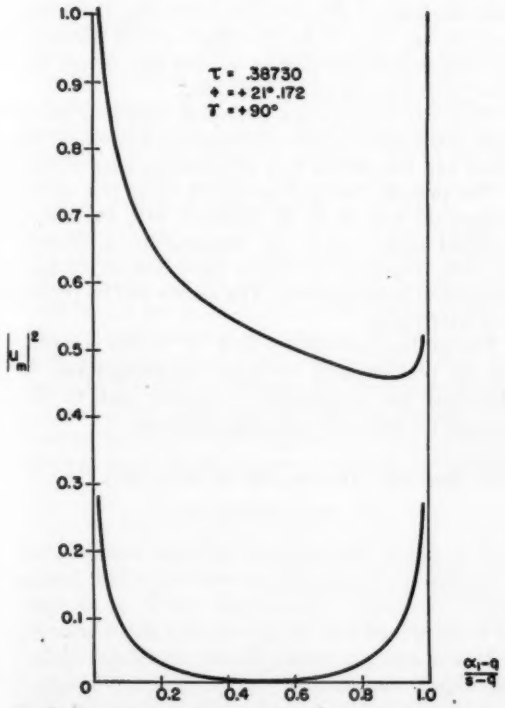

FIG. 2. Metastable light intensity on the bright and dark side, plotted against position of the pinpoint of light within the phase annulus.

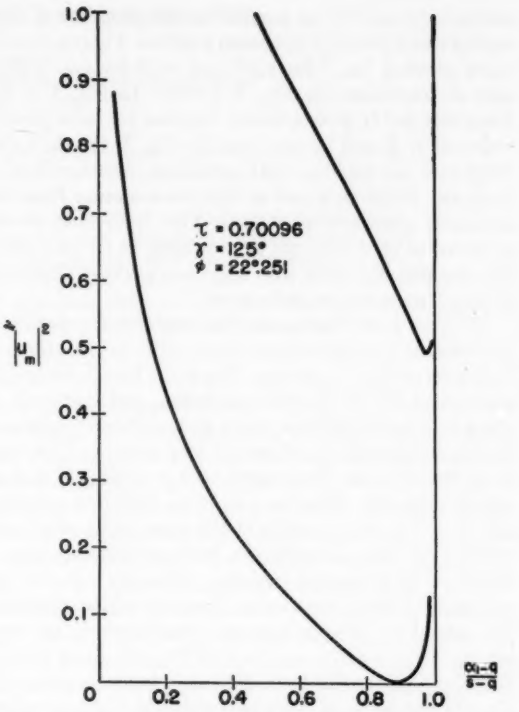

FIG. 3. Analogous to Fig. 2, but showing a misguided attempt to increase the light transmission of the annulus.

Let $\varphi>0$ and $|\varphi|$ small. If the upper sign in Eq. (10) is used, the contrast is said to be negative. If the lower sign is used, the contrast is positive.

Solving Eq. (10) for $\cot\varphi$, substituting into Eq. (8), and simplifying, we have:

$$\ln|(\alpha_1-q)/(s-\alpha_1)|=\pm\pi\cot\gamma \quad (8a)$$

if $|u_m|^2_{\min}=0$.

One obtains from Eq. (10) the false impression that the common practice of using a quarter-wave annulus ($\gamma=\pm 90°$) is unfounded, since then, for small object thicknesses $|\varphi|$, Eq. (10) requires the amplitude transmission τ of the annulus to be small, with resultant difficulty of obtaining adequate illumination. Equation (10) seems to show how, for a given small object thickness $|\varphi|$, optimum contrast can be obtained with high transmission τ if a suitable phase shift γ is chosen. In reality, Eq. (10) is the condition for optimum contrast only if Eq. (8a) is satisfied at the same time, i.e., if the pinpoint of light is in a particular position within the phase annulus. This position is in the middle of the annulus if $\gamma=\pm 90°$, but it rapidly moves away from this position as γ changes. Now if, as is done in practice, the annulus is filled with light (except for a narrow zone along each edge), the greater part of this light goes through a position in the annulus for which contrast is not optimum. It is then important that contrast should

deteriorate as little as possible as the pinpoint of light moves away from the optimum position. Figures 2 and 3 show plots of $|u_m|^2$ for $x>0$ and $x<0$ for two typical sets of conditions. In Fig. 2, $\gamma=90°$. In Fig. 3, γ has been changed to give optimum contrast for some greater value of τ. It will be seen that in Fig. 3, the dark side brightens up rapidly, with resultant deterioration of contrast, as the pinpoint of light moves away from the optimum position; whereas in Fig. 2, so long as the pinpoint of light does not get too close to either edge of the annulus, the dark side brightens up very little and contrast remains generally good.

Thus we have vindicated the established practice of introducing a quarter-wave phase shift in the annulus. It is interesting to note that this is the fourth investigation which has led to this conclusion, and that each of these four investigations starts from different premises. Zernike[7] supposes the object to be a grating. This may be objected to on the ground that a typical biological object does not resemble a grating. Richter[8] supposes the object to be a particle of the same order of magnitude as the limit of resolution. Such particles are indeed common in biological objects, although not to the exclusion of larger structures. Toraldo,[2] while supposing the same kind of object as we, considers only the case where $\ln|(\alpha_1-q)/(s-\alpha_1)|=0$ in Eq. (7), and decides that, for high sensitivity to thin objects, the last term in Eq. (7) should be as large as possible in absolute value. In the light of the work of Rubinowicz,[9] it is clear that conclusions drawn for the present object apply equally to the curved edge of an extended, uniformly phase-shifting object structure, and such structures are indeed common in biological objects.

If $\alpha_1=q$ or $\alpha_1=s$, one of the cosine integral terms in Eq. (3) goes to $-\infty$, while all the others remain finite. It would appear, then, that the intensity $|u|^2$ will be infinite over the entire field of view. In reality, the changes in intensity due to a given object structure are significantly large even at large distances from the object structure itself. It is no longer possible to examine the image of one discontinuity in the object as apart from all others, and since the light imaging all these structures is coherent, the resultant intensity is finite, even though the individual amplitudes are infinite. Among the object structures, one must now include the edge of the illuminated portion of the field of view. Theoretically, an example could be calculated, although not necessarily a typical one, of such a light distribution, by again setting $f=0$ for $|x|>a$ in Eq. (2a). However, the resulting light intensity distribution will bear no resemblance to anything observable with commercial phase contrast equipment, since the latter will always image a point of the light source onto the phase annulus with serious aberrations, and even out of focus. Nonetheless, it is easy to observe in practice that the character of the image changes completely for light passing very close to the edge of the annulus. This was previously found experimentally by J. Magliozzi.[10] Magliozzi's experiments were performed in an attempt to make more light available for illumination by making the phase annulus wider than is done in present Bausch & Lomb phase contrast accessories. If such an annulus is not filled with light to its very edge, the contrast becomes feeble, since the metastable region is no longer well formed. That light which passes close to the edge of such an annulus produces another kind of contrast, which is in the form of a broad halo clinging to every object structure. Of course, light passing close to the edge of the annulus will produce this type of image no matter whether the annulus is broad or narrow. The light which has gone through the interior of the annulus does not contribute to the contrast of this halo, but instead will cover it as with a fog. For optimum contrast as defined in the light of this theory, the annulus should therefore be narrow and should not be filled with light up to its very edge.

Photomicrographs of a biological object to illustrate these phenomena will be published by Butterfield, Magliozzi, and the author in a forthcoming issue of *Science*.

The present theory is currently being put to an experimental test by D. R. Herriott with the aid of an artificial object made by evaporation of fluorite in vacuum. Preliminary results have led us to further theoretical investigations. The results will be published in a later paper.

The author is indebted to L. V. Foster for proposing the problem and for much encouragement, to J. Magliozzi for constructive criticism, and to G. A. Smythe for help with the computations.

[7] F. Zernike, Physica **9**, 686, 974 (1942).
[8] R. Richter, Optik **2**, 342 (1947).
[9] A. Rubinowicz, Ann. d. Physik **53**, 257 (1917).
[10] J. Magliozzi, J. Opt. Soc. Am. **37**, 982A (1947).

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Microscope Imagery and Interpretations*

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The physical limits of resolution are discussed for the following basic types of object specimens: periodic structures which exert no focusing action; periodic structures which do exert focusing action upon the incident light; two pin holes or two small particles against opaque or transmitting backgrounds; two narrow slits or object strips against opaque or transmitting backgrounds; and two, sub-microscopic, self-luminous particles. Abbe's theory remains valid as applied to periodic structures. Generalized resolution formulas are given for periodic structures. Graphical data, based upon recent analysis, show that the resolution of two particles or slits is more complex than indicated by classical theory. The resolving power of the microscope is underestimated by the classical theory. Abnormally high resolutions can occur with properly chosen particles. The class of particles for which the phase microscope has superlative resolving power is described.

I. INTRODUCTION

As is the case with many other physical instruments, the microscope does not provide direct means for measuring the properties of the object. Instead, quantitative information about the object has to be deduced from the microscopic data. In order to make greater quantitative use of the microscope, we must become more skilled in the interpretation of the diffraction image of various object specimens.

Although the essential physical principles and much of the required analytical techniques were known in 1880, and even in 1850, yet to this date, semirigorous interpretations are known for but a few simple types of object specimens. In the year 1949 we have still to complete the elementary problems whose solutions were begun by Airy, continued by Abbe and neglected by the modern physicist. However, the last decade has witnessed a revival of interest in the interpretation of microscopic imagery. Notable contributions to the diffraction theory of optical systems have been made during recent years in both America and Europe.

It is the purpose of this paper to review, as far as space permits, the laws of resolution of the simplest object specimens against dark and bright backgrounds. Accordingly, a presentation of the theoretical evidence for the new conclusions is not possible. In brief, most of the new conclusions have been reached by taking into account the interaction between the Huygens wavelets which arise at the object particle and its surround. This interaction is included automatically in the diffraction integrals of phase microscopy or of ordinary microscopy.

II. NOTATION

The required notation is illustrated in Fig. 1. It is supposed that a continuous, extended lamp filament is focused as in Kohler illumination upon a condenser diaphragm which is located at the first focal plane of the sub-stage condenser. Let P denote a point located at the *edge* of the opening in the condenser diaphragm. An axial ray is traced from P through the system. This ray makes the polar angle ϑ_{cm} with the optical axis in the object space. The selected numerical aperture of the condenser is equal to the product of the refractive index n_0 of the object space and $\sin\vartheta_{cm}$. If the condenser diaphragm is opened far enough, the point P moves outward to the point P_m for which the angular opening ϑ_{cm} of the broken axial ray just fills the objective. This particular value of ϑ_{cm} is denoted by ϑ_{om}. The numerical aperture $n_0 \sin\vartheta_{om}$ of the condenser is now equal to the numerical aperture of the objective which is defined variously in the literature by Eq. (1). As the condenser diaphragm is opened from a pin hole on the optical axis to a circle of sufficiently large radius, the object specimen is illuminated by cones of *axial illumination* whose numerical aperture varies from zero to the numerical aperture of the objective. The corresponding S ratio is defined by Eq. (3).

$$\text{N.A.}_{\text{objective}} \equiv |M| \sin\vartheta_m \equiv |M| \rho_m = n_0 \rho_{om}. \quad (1)$$

$$\text{N.A.}_{\text{condenser}} \equiv n_0 \sin\vartheta_{cm} \equiv n_0 \rho_{cm}. \quad (2)$$

$$S \equiv \frac{\text{N.A.}_{\text{condenser}}}{\text{N.A.}_{\text{objective}}} = \rho_{cm}/\rho_{om}. \quad (3)$$

The same notation applies to oblique illumination. If a small hole is drilled in an opaque diaphragm about the point P, the object plane is illuminated by an oblique pencil of rays whose numerical aperture is defined by the product $n_0 \sin\vartheta_{cm}$ and whose S value is defined by Eq. (3).

We will state how resolving power depends upon the ratio S of the numerical apertures in the restricted range $0 \leqq S \leqq 1$. It is pointed out that whereas this restriction of the S range is customary, this restriction is somewhat arbitrary. In principle, resolving power can be computed also in the case $S > 1$, that is in the case in which the numerical aperture of the condenser exceeds that of the objective. That resolving power continues to vary beyond the point $S = 1$ can be suspected from a cursory examination of the resolution formulas.

* Presented as an invited paper at the Buffalo meeting of the Optical Society of America (October, 1949).

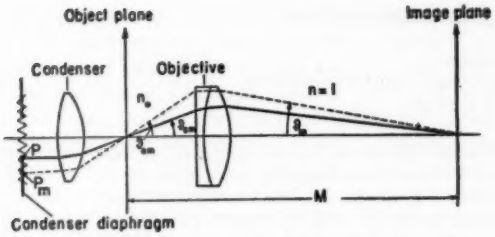

FIG. 1. Notation with either axial or oblique illumination.

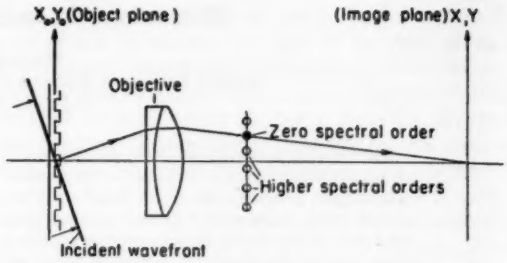

FIG. 2. Imagery with periodic object structures which exert no focusing action upon the incident light. The spectral orders ν, μ are focused in the second focal plane of the objective as images of the point in the source from which the incident wave front is radiated.

III. RESOLUTION OF PERIODIC STRUCTURES WHICH EXERT NO FOCUSING ACTION ON THE INCIDENT LIGHT

Figure 2 illustrates a non-self-luminous, periodic object which exerts no focusing action upon the incident illumination. The elevations and troughs may represent optical path differences or differences in light transmission or both. We suppose that these elevations and troughs are arranged on a lattice whose spacing along X_0 and Y_0 is $2l$ and $2h$, respectively. The amplitude and phase transmission of such a crossed grating can be represented by a double Fourier series whose frequency numbers ν, μ range from $-\infty$ to $+\infty$. It can be shown that the frequency numbers ν, μ enumerate the spectral orders produced by the crossed grating. The existence of these spectral orders can be demonstrated from Huygens' construction Each radiator in the source of light gives rise in this manner to a separate set of spectral orders. Only those spectral orders which are not too steeply inclined from the optical axis will be intercepted and transmitted by the objective. It was Abbe's contention that the image of the object is determined by the subsequent interference of the transmitted spectral orders in the image plane. Abbe concluded that the coarsest detail in the object structure can be resolved if and only if the grating spacing, the inclination of the incident wave front and the numerical aperture of the objective are so related that the zero order and at least one spectral order on at least one side of the zero order are transmitted by the objective. Lummer added that the image and object become similar as the number of transmitted spectral orders becomes great.

At least the most important conclusions drawn by Abbe and Lummer can be verified by Fourier analysis within the physical scope of the Luneberg-Debye[1] diffraction formula. This analysis leads to a more general resolution formula than that deduced by Abbe. This resolution formula is given by Eq. (4) in which, to repeat, $2l$ and $2h$ are the separations in wave-lengths of the crossed-object structure and the positive and negative integers ν, μ denote the spectral orders. Equation (5) is the resolution formula for the simply periodic grating. It is obtained from Eq. (4) by supposing that the separation $2h$ becomes great.

$$\frac{4lh}{(\nu^2h^2+\mu^2l^2)^{\frac{1}{2}}} \geqq \frac{2}{\text{N.A.}_{\text{objective}}+\text{N.A.}_{\text{condenser}}} \text{ wave-lengths.} \quad (4)$$

$$2l \geqq \frac{|\nu|}{\text{N.A.}_{\text{objective}}+\text{N.A.}_{\text{condenser}}} \text{ wave-lengths.} \quad (5)$$

The first harmonics for crossed-periodic structures correspond to the choice of $\nu=0$, $\mu=\pm1$ or of $\mu=0$, $\nu=\pm1$ in Eq. (4). The first harmonic for simply periodic structure corresponds to the choice of $\nu=\pm1$ in Eq. (5). The choice of the greater-than sign gives the physical requirement for the presence of the ν, μth spectral harmonic in the image of the periodic object structure. In applying Eqs. (4) or (5) for the purpose of ascertaining the resolving power of the objective, it is customary to check only for the presence of the first spectral harmonics. According to Lummer's principle, a more complete test for the definition possible with a given objective will be obtained by finding the *highest* spectral harmonic which satisfies Eqs. (4) or (5).

The following phenomena are emphasized here because they have been a source of much confusion.

First, the choice of the equality sign gives the *physical limit* of resolution of the ν, μth spectral harmonic. This is a true limit which is approached but never attained by a physical receptor of the energy density in the image no matter how sensitive is the receptor to differences in contrast.

Secondly, the choice of the *greater-than sign* in Eqs. (4) and (5) is a necessary but not a sufficient condition for resolution. The amount by which the left-hand member must exceed the right-hand member depends upon the composition of the object structure, upon the aberrations of the objective, upon the chosen cone of illumination and upon the particular system of microscopy. In general, the physical limit will be approached more closely by a variable phase microscope than by the classical bright field microscope.

[1] R. K. Luneberg, *Mathematical Theory of Optics* (Brown University, Providence, 1944), p. 364.

Thirdly, the sum of the numerical apertures of the objective and condenser belongs for mathematical reasons, as shown, in the resolution formula for periodic structures. We shall see later that this is not true in the case of an object which consists of two particles.

Fourthly, Eqs. (4) and (5) apply to self-luminous periodic structures if the numerical aperture of the condenser is set equal to the numerical aperture of the objective. Image formation with luminous and non-self-luminous objects is, however, quite different in other respects.

Fifthly, Eqs. (4) and (5) govern *both* axial and oblique illumination. Axial illumination contains all possible pencils of oblique illumination within a cone of given numerical aperture. Hence Eqs. (4) and (5) will be satisfied for a greater number of spectral orders with a given cone of axial illumination than with a single pencil of oblique illumination. However, contrast in the image may be better with a suitably chosen pencil of oblique illumination or with a suitably modified cone of axial illumination. Hollow cones produced by annular openings in the condenser diaphragm are often advantageous. There is considerable amount of truth in Abbe's statement that a full cone of axial illumination "spoils" the image.

Sixthly, a distinction should be drawn between contrast and resolution. Since an object specimen must be seen in order to be resolved, the physical limits of resolution may be approached more closely by the optical system which is capable of the greater contrast. It is important to appreciate that Eqs. (4) and (5) can be derived irrespective of the aberrations of the objective and of the nature of the source of light provided only that the source of light is continuous in a system employing Kohler illumination. The primary effect of lens aberrations, of the choice of light source and of the choice of opening in the condenser diaphragm is to alter contrast in the image of periodic object structures. It follows as an interesting corollary that the physical limits expressed by Eqs. (4) and (5) cannot be reduced by any physical means other than decreasing the wave-length of the incident light or increasing the numerical aperture of the objective. The phenomena which may take place when the numerical aperture of the substage condenser exceeds the numerical aperture of the objective are beyond the scope of the present discussion.

IV. RESOLUTION OF PERIODIC STRUCTURES WHICH EXERT FOCUSING ACTION ON THE INCIDENT LIGHT†

Since most biological particles exert some focusing action upon the incident light, there exists *a priori* the danger that the omission of such focusing action in a theory of resolution of periodic structures may lead to conclusions which do not agree with experiments upon biological specimens, for example, diatoms. The presence of focusing action makes the resolution problem more difficult, but it turns out that the problem can nevertheless be solved formally without approximation. Curiously enough, the solution leads to two, almost independent, resolution formulas given by Eqs. (6) and (7). Both of these formulas have to be considered in determining how well the object structure is resolved.

Suppose that the object consists of refracting spherical or lens-like particles which are arranged on a lattice in the X_0', Y_0'-plane as in Fig. 3. For a given incident wave front, the light which is *intercepted* by the spherical particles is focused paraxially in a definite X_0, Y_0-plane as a lattice arrangement of diffraction images of the point in the source of light. It can be shown that this lattice arrangement of diffraction images gives rise to its own set of spectral orders. These spectral orders are denoted by the numbers $\pm m$ and $\pm n$ and are governed by the resolution formula of Eq. (7) in which $2l$ and $2h$ are again the lattice spacing in wave-lengths along X_0 and Y_0, respectively.

$$\frac{4lh}{(\nu^2h^2+\mu^2l^2)^{\frac{1}{2}}} \geqq \frac{2}{\text{N.A.}_{\text{objective}}+\text{N.A.}_{\text{condenser}}} \text{ wave-lengths.} \quad (6)$$

$$\frac{4lh}{(m^2h^2+n^2l^2)^{\frac{1}{2}}} \geqq \frac{2}{\text{N.A.}_{\text{objective}}+\text{N.A.}_{\text{condenser}}} \leqq \frac{2}{\text{N.A.}_{\text{particles}}}. \quad (7)$$

The first significant fact about the spectral orders m, n is that they are finite in number as they leave the object plane and are limited in number by the numerical aperture of the object particles which act as lenses. The first and last members of Eq. (7) express this fact analytically. For the usual biological spheres the numerical aperture will rarely exceed 0.1 and will usually be 0.05 or less. This means that there will be few spectral orders m, n unless the separations $2l$ and $2h$ are large. There may in fact be no spectral orders at all beyond the zero order. The second significant fact about these spectral orders is that once the sum of the numerical apertures of the objective and condenser equals or exceeds the numerical aperture of the particles, any further increase in the numerical aperture of the objective or condenser will not improve the resolution of these spectral orders. This fact is also expressed by Eq. (7).

Fortunately for the resolution of lattice arrangements of spherical particles, these particles can generate a second and more usual set of spectral orders *provided that the particles are not supported in an opaque back-*

† The dagger will be used hereafter to indicate propositions which have been deduced recently by the writer and which are presumed to be new.

ground. It will be noted from Fig. 3 that each particle cuts a hole in the incident wave front. This hole is projected as an elliptical shadow on the X_0, Y_0-plane. It is not surprising that these shadows should generate a potentially infinite set of spectral orders ν, μ whose resolution is governed by a general equation of the type Eq. (6) which contains, as we have seen, the specialized cases discussed by Abbe and Lummer.

An extremely interesting and fundamentally important situation arises when we suppose that the spherical particles are mounted in an opaque slide such that no scattering of light occurs at the edge of the mount. In this case no shadows are cast upon the X_0, Y_0-plane so that no spectral orders of the type ν, μ can be generated. Within the scope of the Luneberg-Debye formulation of Kirchhoff's law, we obtain only the limited set of spectral orders m, n which are generated by the lattice arrangement of diffraction images formed by the particles of the source of light. We have noted that for these special spectral orders no further improvement in resolution can occur once the numerical aperture of the objective equals or exceeds the numerical aperture of the particles. If, for example, the numerical aperture of the particles is 0.1, no further improvement in resolving power of the optical system will be obtained by the use of objectives of numerical aperture higher than 0.1. Now this prediction either will or will not agree with experiment. It is not beyond conjecture that a carefully controlled experiment may reveal that the resolving power of an optical system continues to improve even when the numerical aperture of the objective is increased beyond that of the particle. If so, the hypotheses or approximations involved in the Luneberg-Debye formulation are not adequate for predicting image formation with spherical particles which are set in an opaque slide. One phenomenon which has been too difficult to include in the Luneberg-Debye formulation is the irregularity or "edge wave" which may be present at the edge of the wave fronts as these wave fronts emerge from lens-like particles.

It would be impractical to attempt a controlled experiment upon a lattice arrangement of many lens-like particles mounted in an opaque slide. Actually, only two particles are required in a critical experiment; for it can be shown† that the resolution of two lens-like particles which are mounted in an opaque slide should not be improved by increasing the numerical aperture of the objective beyond the numerical aperture of the particle.

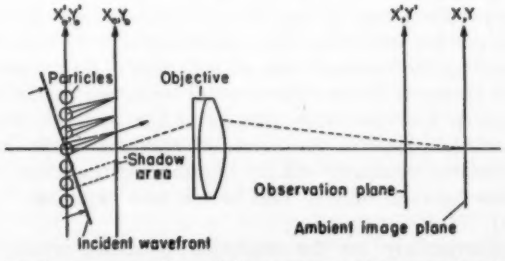

FIG. 3. Imagery with periodic structures which exert focusing action upon the incident light. The location of the conjugate, ambient object and image planes X_0, Y_0 and X, Y, respectively, depends in general upon the inclination of the incident wave front.

V. THE AIRY AND THE PHYSICAL LIMIT OF RESOLUTION

It has been customary to state the resolving power of an optical system for two particles in terms of a limit of resolution which is usually attributed to Airy and which is defined by the equation

$$\text{Airy limit} = \frac{0.6098}{\text{N.A.}} \text{ wave-lengths} \equiv r_a^0 \qquad (8)$$

wherein N.A. denotes the numerical aperture of the objective. Let r denote the separation of the particles at the classical limit of resolution. The accepted resolution formula is then

$$r = \frac{2\times 0.6098}{\text{N.A.}_{\text{objective}} + \text{N.A.}_{\text{condenser}}} \text{ wave-lengths.} \qquad (9)$$

Eq. (9) embodies the classical notions that the limit of resolution is numerically equal to the Airy limit when the numerical aperture of the condenser is equal to the numerical aperture of the objective and that the limit of resolution is twice the Airy limit when the numerical aperture of the condenser is substantially zero.

A second classical formula for the limit of resolution, r, is given by

$$r = \frac{1}{\text{N.A.}_{\text{objective}} + \text{N.A.}_{\text{condenser}}} \text{ wave-lengths.} \qquad (10)$$

A comparison of Eqs. (5) and (10) shows that Eq. (10) is simply an extension of the physical limit of resolution for periodic structures to the quite different case of two particles.

The Airy limit is defined in terms of a very special diffraction image of a single sub-microscopic particle. The energy density $G(x)$ in this diffraction image is produced by an objective of the Airy type and is given by

$$G(x)/\pi^2\rho_m^4 \equiv g(x) = [2J_1(2\pi\rho_m x)/2\pi\rho_m x]^2. \qquad (11)$$

ρ_m is the numerical aperture of the objective with respect to its image space and is related to the usual N.A. of the objective by the equation

$$|M|\rho_m = \text{N.A.} \qquad (12)$$

in which M is the magnification ratio, J_1 is a Bessel function of the first kind and first order. In an actual case the two particles are rarely sub-microscopic. Furthermore, the objective may not approximate the Airy type closely enough. The main objection to the use of Airy's criterion is that its meaning, as applied to

actual microscopic particles and objectives, becomes too ambiguous for the purposes of analysis and interpretation. One feature of the Airy limit remains valuable. The Airy limit is a conveniently chosen unit of length in terms of which the actual laws of resolution may be expressed universally in the sense that a single computation suffices for objectives of any numerical aperture. Let Eq. (8) define the Airy unit of length with respect to the object space and let

$$r_a \equiv (0.6098)/\rho_m \tag{13}$$

define the Airy unit of length with respect to the image space.

A true physical limit of resolution for two particles has been suggested by C. M. Sparrow and discussed by Ramsay, Cleveland, and Koppius.[2] This limit is amenable without ambiguity to analysis even when the area of the particles and the aberrations of the objective cannot be neglected. Let $G(x)$ denote the energy density in the image of two particles or of two object slits. Let the X-direction be chosen so as to pass through the centers of the images of the two particles or so as to pass perpendicularly through the centers of the images of the two, long object slits. Suppose, for convenience, that the centers of the particles are equidistant from the optical axis. Suppose also that the two particles or object slits are identical. Then

$$dG(x)/dx=0 \tag{14}$$

at $x=0$. If the particles are far enough apart, the energy density $G(x)$ can show a dip at $x=0$ as in Fig. 4. If a dip is present, $[(d^2G(x))/dx^2]\neq 0$ at $x=0$. The separation of the two particles may now be reduced to the value for which

$$[d^2G(x)]/dx^2=0 \tag{15}$$

at $x=0$. At this separation of the two particles a true physical limit of resolution is reached. There will be no dip to be detected in the energy density *along the X-direction* no matter how sensitive the detector. We shall call the separation of the two particles for which Eqs. (14) and (15) are satisfied the physical limit of resolution for two particles and shall express this separation in Airy units.

Suppose that two dissimilar particles are located equidistantly from the optical axis. The point $x=x_p$ at which $[dG(x)]/dx=0$ is then to be discovered. The physical limit of resolution of such particles is now the separation for which

$$[d^2G(x)]/dx^2=0 \tag{16}$$

at the point $x=x_p$. It can happen with suitably chosen dissimilar particles that $x_p=0$.

The reader will be correct in surmising that the physical limit is always tedious and frequently difficult to compute.

[2] Ramsay, Cleveland, and Koppius, J. Opt. Soc. Am. 31, 28 (1941).

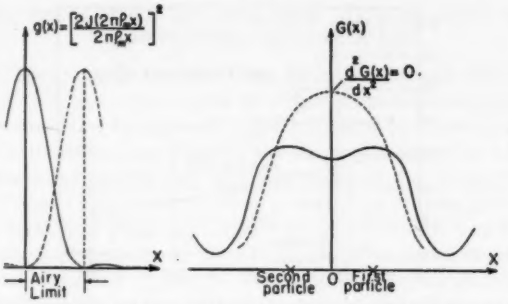

FIG. 4. Comparison of the Airy and the physical limits of resolution. The unbroken curve of the right-hand drawing represents the energy density $G(x)$ in the image of two, like particles whose separation is greater than the physical limit. The broken curve represents $G(x)$ when the separation of the particles is equal to the physical limit of resolution. Whereas the energy density $g(x)$ is specialized, the energy density $G(x)$ is not.

Before passing to the next sections which apply to two non-self-luminous particles or slits, we state the physical limit of resolution for two, sub-microscopic, self-luminous particles. This physical limit is 0.78 Airy unit for objectives of the Airy type and is a better approximation than Luneberg's[3] value 0.8. Consequently, the physical limit of resolution for two self-luminous particles is 22 percent smaller than the classical Airy limit. For comparison with the various physical limits computed for non-self-luminous particles, the above physical limit is marked with an X in the following figures. We shall see that the physical limit for non-self-luminous particles can be lower than the physical limit for self-luminous particles. The classical notion that self-luminous particles are the most easily resolved particles does not rest upon firm theoretical foundations. We shall see, moreover, that the resolution formula of Eq. (9) is at best the first approximation to the truth. In general, the microscope is theoretically capable of greater resolution than is predicted by Eq. (9). It is pertinent to remark that there exists today a growing conviction, based upon experiment, that the microscope is capable of greater resolution than predicted by Eq. (9).

VI. RESOLUTION OF TWO LIKE PIN HOLES IN AN OPAQUE SLIDE

In Fig. 5 are plotted the separations $2L$ in Airy units of two like pin holes in an opaque slide at the Abbe limit and at the physical limit of resolution† as a function of S, the ratio of the numerical aperture of the condenser to the numerical aperture of the objective. The Abbe limits have been obtained from Eq. (9). It will be supposed henceforth for definiteness that the microscope objective is of the Airy type. In computing the physical limits, it has been supposed that the effective radius of the pin holes is less than one-half Airy unit. Provided that such pin holes have equal

[3] R. K. Luneberg, reference 1, p. 390.

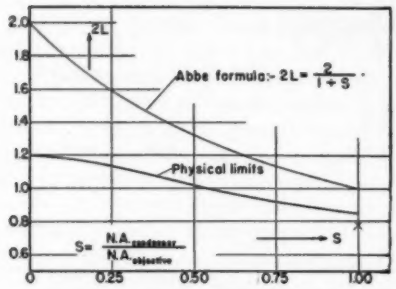

FIG. 5. Comparison of the Abbe limits and the physical limits as functions of S in resolving two like pin holes in an opaque slide. $2L$ is the separation of the two particles in Airy units.

areas and have like amplitude and phase transmission, it can be shown that the physical limits are independent of the area of the particles.

The main conclusions to be drawn from Fig. 5 are: (1) the curve for the Abbe limits is everywhere above the curve for the physical limits; (2) whereas the two sets of limits agree rather well near $S=1$, the Abbe curve departs markedly at low values of S; (3) the Abbe curve predicts too rapid increases in resolving power with increasing openings in the condenser diaphragm; (4) the physical limits for this type of particle are higher than the physical limit for two self-luminous particles.

The following remark is significant. If one assumes that dips of 3 to 5 percent in the energy density are detectable, isolated calculations indicate that the presence of two particles should be ascertained when their separations $2L$ fall much nearer to the curve of the physical limits than to the curve of the Abbe limits. As a corollary, the closeness of approach of the actual resolution to the physical limit is a measure of the least detectable dip in the curve of the energy density.

VII. RESOLUTION OF TWO UNLIKE PARTICLES AGAINST AN OPAQUE BACKGROUND†

Whereas Fig. 5 illustrates a mild sort of departure from Abbe's theory, the departures of Fig. 6 are impressive. The departures are again in the direction of better resolution. Plotted here are the separations $2L$ of the particles as a function of S for two pin holes coated so that their optical path difference has the indicated values Δ. The case $m=1$ is that in which the product of the area and the amplitude transmission of one pin hole is equal to the same product for the second pin hole.

If the optical path difference between the two pin holes is less than one-quarter wave-length, the physical limits are improved by opening the condenser diaphragm, that is by increasing S.

If the optical path difference is one-quarter wave-length, the opening in the condenser diaphragm has no effect upon the physical limits.

If the optical path difference exceeds one-quarter wave-length, opening the condenser diaphragm has a deteriorating effect upon the physical limits of resolution. We learn that there can exist pairs of unlike particles for which the condenser diaphragm should be set at the smallest openings consistent with adequate illumination in order to obtain the best possible resolving power. Thus far, this peculiar anomaly has been found to be true only in the case of two unlike particles whether these particles are set in an opaque or in a transmitting background.

The reference cross falls upon the curve drawn for $\Delta=90°$. We note that there can exist non-self-luminous particles which are more readily resolved than are two self-luminous particles.

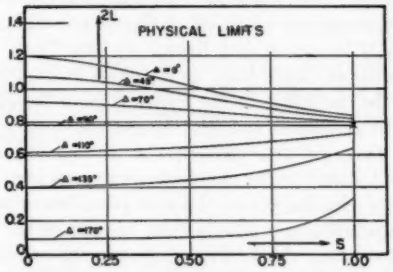

FIG. 6. Separations $2L$ in Airy units at the physical limits of resolution of two unlike particles against an opaque background. Δ is the optical path difference between the two particles. The two particles are restricted to the class $m=1$ in which the product of the area and the amplitude transmission of one particle is equal to the same product for the second particle.

Particles having the greater optical path differences are the most readily resolved.

The trends of Fig. 6, particularly when $\Delta>90°$, are sufficiently radical to suggest the possibilities that the computations for the physical limits are erroneous or that the physical limits are not necessarily a good indication of the attainable resolution with two particles. A series of diffraction curves were drawn therefore of the energy density $G(x/r_a)$ for different values of $2L$ and Δ. These diffraction curves proved to be consistent with the trends of Fig. 6. For example, with $\Delta=90°$ and $2L=0.9$ the curves $G(x/r_a)$ were identical for all values of S. The dip in the energy density at $x/r_a=0$ was 10 percent of the maximum value which occurred at $x/r_a=0.43$. The cases $\Delta=70°$ and 135° at the separation of 0.9 Airy unit are included as Figs. 7 and 8, respectively. Figure 7 shows that the particles are unresolved when $S=0$ but that resolution may be possible when $S=1$. This is clearly consistent with Fig. 6. Figure 8 shows that the particles are easily resolvable at $S=0$ but that the two particles are much less resolvable when the numerical aperture of the condenser is increased to the point $S=1$. These phenomena are consistent with Fig. 6. The physical reasons why two pin holes in an opaque slide should be more readily resolved as their optical path is increased to one-half wave-length are not entirely clear. It is not surprising, however, that the energy density should approach zero at

the midpoint $x=0$ in the image as Δ approaches one-half wave-length.

The main conclusions to be drawn from this section are: (1) The resolving power of a microscope for two particles depends in general upon the area and upon the phase and amplitude transmission of the particles; (2) the Abbe theory for the resolution of two particles is to be regarded with great skepticism although Abbe's notions were substantially correct with respect to the resolution of objects having periodic structure; (3) no simple resolution formula of the Abbe type holds in the case of two particles.

VIII. RESOLUTION OT TWO, LIKE, NARROW SLITS AGAINST AN OPAQUE BACKGROUND†

The physical limits for two, like, narrow, transmitting slits in an opaque slide are compared in Fig. 9 with the physical limits for two particles against an opaque background. It has been supposed that the two object slits are illuminated by light issuing from a condenser diaphragm whose opening is a slit oriented at right angles to the object slits. This type of illumination will be called lateral slit illumination. The S ratio is

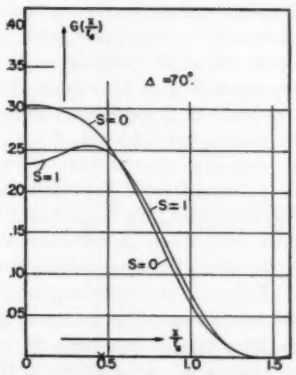

FIG. 7. Comparison of diffraction curves obtained with $S=0$ and with $S=1$ in the case of two unlike particles (class $m=1$) whose optical path difference is 70° and whose separation is 0.9 Airy unit. When $S=1$, a maximum appears in the neighborhood of $\pm x/r_a=0.45$. The particles are unresolved at the condenser setting $S=0$.

now determined by the length of the slit in the condenser diaphragm.

It will be seen that two such object slits are more easily resolved than are two particles. The relative position of the cross indicates that two slits in an opaque background may be resolved more readily than are two self-luminous particles. The difference between the physical limits of two slits and two particles is not, however, great. Occasionally one hears the statement that two slits are resolved twice as well as two particles. This statement is not in accord with the two curves of Fig. 9.

IX. RESOLUTION OF TWO LIKE SLITS AGAINST A TRANSMITTING BACKGROUND†

The physical limits of Fig. 10 have been calculated for two, like, narrow slits or object strips located on a transmitting background and illuminated by lateral slit illumination. The width of the strips is assumed to be less than one-half Airy unit. The curves of Fig. 10 apply to object strips which differ in optical path from their surround by either zero or one-half wave-length. Let W denote the width of the object strips in wave-length numbers. Let g denote the ratio of the amplitude transmission of the strip to the amplitude transmission of the surround. When the optical path difference between strip and surround is zero, the product fk_1 is given by

$$fk_1=(g-1)(\text{N.A.})W. \tag{17}$$

When the optical path difference is one-half wave-length,

$$fk_1=-(g+1)(\text{N.A.})W. \tag{18}$$

It can be expected that the more usual fk_1-values will fall in the range $-0.25\leqq fk_1\leqq 0$. The two curves of Fig. 10 belong to the extremes of this range.

Two narrow object strips on a bright field are resolved more readily than two slits in an opaque slide. When the numerical aperture of the condenser approaches the numerical aperture of the objective, the two object strips have distinctly lower physical limits than given by the reference cross for two self-luminous particles. Since the physical limits vary with fk_1, the resolution of two object strips against a transmitting background depends upon the width and the optical properties of the strip relative to the optical properties of the surround.

X. RESOLUTION OF TWO LIKE PARTICLES AGAINST A TRANSMITTING BACKGROUND†

The two particles shall be substantially uniform in amplitude and phase transmission and shall not overlap. They may be located in a transparent slide. Their effective radius shall not exceed one-half Airy unit.

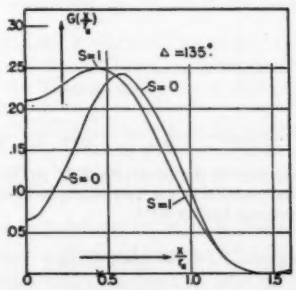

FIG. 8. Comparison of diffraction curves obtained with $S=0$ and $S=1$ for two particles (class $m=1$) whose optical path difference is 135° and whose separation is 0.9 Airy unit. When $S=1$, the first maxima occur near $\pm x/r_a=0.45$. When $S=0$, the first maxima are much more pronounced and occur near $\pm x/r_a=0.59$ Airy unit.

The four curves of Fig. 11 belong to particles whose optical path is either equal to that of an equal thickness of the surround or differs from that of the surround by one-half wave-length. When the optical path difference is zero,

$$fk=-(1-g)\pi(\text{N.A.})^2A. \tag{19}$$

When the optical path difference is one-half wavelength,

$$fk=-(1+g)\pi(\text{N.A.})^2A. \tag{20}$$

A is the area of each particle in number of square wavelengths. g is again the ratio of the amplitude transmission of the particle to the amplitude transmission of the surround. N.A. is the numerical aperture of the objective. For particles of the above category, the dependence of the physical limits upon the opening in the condenser diaphragm has been ascertained by a relatively slight modification of Eq. (25) in a paper by Osterberg and Wissler.[4]‡

The curves of Fig. 11 show that the physical limits depend upon the products fk and hence upon the dimensions and the optical properties of the two particles. In the neighborhood of $S=1$ the physical limits for two non-self-luminous particles can become markedly smaller than the physical limit for two self-luminous particles. In general, two particles on a bright field are resolved less readily than two object strips on a bright field. This statement follows from a comparison of Figs. 10 and 11. A comparison of Figs. 5 and 11 shows that two like particles in a bright field are more readily resolved than two like particles against an opaque background.

It may be stated as a generalization that the physical limits for two like particles or object strips are monotone decreasing functions of the numerical aperture of the condenser in the range $0 \leqq S \leqq 1$. Furthermore, when the S ratio has reached the point $S=0.75$, a negligible gain in resolution is obtained by increasing S toward unity. The growing practice of setting the numerical aperture of the sub-stage condenser near three quarters of the numerical aperture of the objective is therefore to be justified whenever an S setting of 0.75 is accompanied by increased contrast in the image. The curious phenomenon in which the physical limits increase with the numerical aperture of the condenser occurs only with certain classes of *unlike* particles against an opaque or transmitting background. Probably no one has as yet had the fortune or misfortune of viewing two such particles in a microscope.

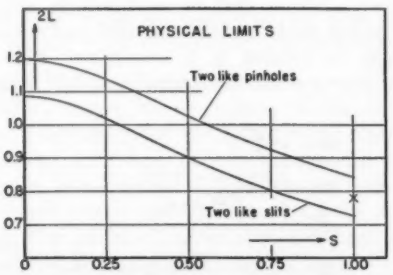

FIG. 9. Comparison of the separations $2L$ in Airy units at the physical limits of resolution for two like pin holes and for two like slits in an opaque background.

XI. RESOLVING POWER OF THE PHASE MICROSCOPE†

One group of microscopists often ask the question "Can the phase microscope resolve as well or better than the ordinary microscope?" A second group is more likely to ask "How much is the resolving power of an objective decreased in a system of phase microscopy?"

The phase microscope was intended originally for the purpose of increasing contrast in the image of a particle whose optical path difference Δ with respect to its surround is so small that the particle is either invisible or appears in poor contrast in the classical microscope. For the purpose of resolving two such particles the phase microscope is fundamentally superior to the ordinary microscope. It can be shown that when the optical path difference Δ between the particles and surround is so small that $\sin\Delta=\Delta$, no physical limits exist for the ordinary microscope whereas physical limits can be computed from Eq. (15) for the phase microscope. Physical limits for the ordinary microscope begin to appear only when Δ becomes so large that powers in Δ higher than the first cannot be neglected in the expansion of $\sin\Delta$ or $\cos\Delta$ into a power series in Δ. This conclusion is not surprising, for two particles must be seen before they can be resolved. It is surprising, however, that when $\sin\Delta$ approaches Δ, the resolving power of the phase microscope depends primarily upon the size and shape of the opening in the condenser diaphragm and only secondarily upon the coating materials of the diffraction plate. The primary effect of the

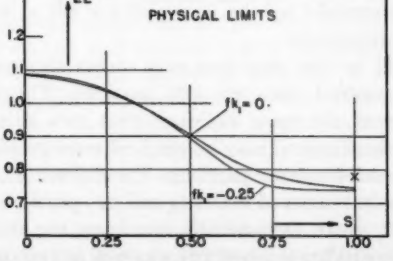

FIG. 10. Separations $2L$ in Airy units at the physical limits of resolution of two object slits against a transmitting background. See Eqs. (17) and (18) for the meaning of fk_1.

[4] H. Osterberg and F. C. Wissler, J. Opt. Soc. Am. 39, 561 (1949).

‡ The modification consists of integrating with respect to $d\rho$ from $\rho=0$ to $\rho=S\rho_m$ rather than from $\rho=0$ to $\rho=\rho_m$ in Eq. (25). The condition of Eq. (31) for the physical limits becomes more complex and involves S as an additional parameter. The results of this paper can be modified so as to apply also to particles in an opaque background and to object slits in a transmitting or opaque background.

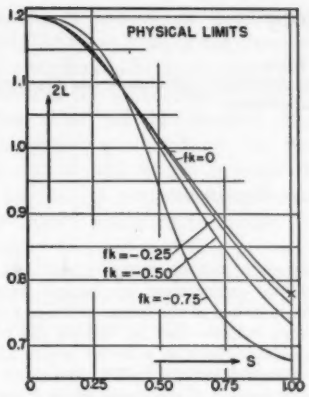

FIG. 11. Separations $2L$ in Airy units at the physical limits of resolution for two like particles against a transmitting background. See Eqs. (19) and (20) for the meaning of fk.

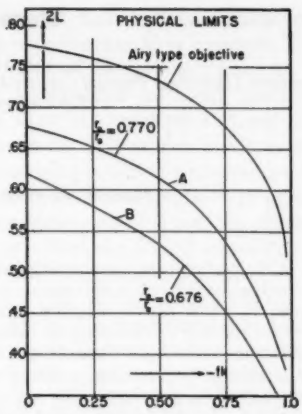

FIG. 12. The separations $2L$ in Airy units at the physical limits of resolution as functions of fk at $S=1$ for two like particles against a transmitting background. The three comparison curves belong to special objectives whose exit pupils have been coated with refracting and absorbing materials for the purpose of controlling the diameter of the central or Airy diffraction disk.

coating material is to modify contrast in the image of the two particles. The physical limits of a phase microscope for two particles of the above category can fall near or below 0.78 Airy unit.

It should not be concluded from the above remarks that the resolving power of a phase microscope may be superior to that of the ordinary microscope for other classes of particles. Resolving power depends in a complicated manner upon the combined dimensional and optical properties of the optical system and of the object specimen. Generalizations such as the classical Eq. (9) are to be avoided at the present time.

XII. EFFECT OF SPECIAL COATINGS UPON THE PHYSICAL LIMITS OF RESOLUTION FOR TWO PARTICLES

The resolution of two particles differs in an interesting fundamental respect from the resolution of periodic structures. Whereas it is physically impossible within the scope of the Luneberg-Debye diffraction integrals to decrease the physical limits below those already mentioned for objects of periodic structure, it is theoretically possible to coat the exit pupil of an objective with refracting and coating materials so as to reduce the physical limits of resolution for two particles below the limits attainable with the classical Airy type objective. It is in principle within the possibilities of the modern reflecting objective to accomplish this result to a limited degree.

In Fig. 12 are plotted the physical limits for two particles for three different types of objectives as a function of fk (see Eqs. (19) and (20)). The numerical aperture of the condenser is assumed to be equal to the numerical aperture of the objective. The upper curve has been plotted for an Airy type objective. Curve A has been plotted for an objective whose exit pupil has been coated so as to bring the location of the first diffraction minimum from one Airy unit to 0.77 Airy unit. Similarly, the objective of curve B has been treated so as to reduce the ratio r_0/r_a from one Airy unit to 0.676 Airy unit.

It will be noted that the physical limits for the specially coated objectives fall well below the physical limits for the Airy type objective. As might be expected, a penalty is incurred in attempting to lower the physical limit of resolution. This penalty[5] involves a marked loss of contrast in the diffraction image.

XIII. SUMMARY

In summary, the following observations are of main interest.

(1) Abbe's conclusions about the resolution of objects having periodic structure are substantially correct and can be regarded as specializations of a more general formulation. This more general formulation includes objects whose periodic structure can exert focusing action upon the incident light.

(2) On the other hand, the resolution of two particles or two slits located on an opaque or transmitting background is much too complex to be represented by the resolution formula attributed to Abbe.

(3) The resolutions of two particles or slits by an Airy type objective is better than indicated by prior classical theory and depends upon the dimensions and the optical properties of the particles or slits.

(4) Finally, resolution depends upon the particular system of microscopy. Thus the resolving power of a phase microscope can be expected to exceed that of the usual microscope in the case of particles for which the phase microscope was originally intended.

The author is indebted to Miss Barbara R. Kloepfer for calculating the physical limits and diffraction curves for two particles and to Robert A. McDonald for calculating the physical limits for two object slits.

[5] H. Osterberg and F. C. Wissler, J. Opt. Soc. Am. 39, 565 (1949).

Infra-Red Microspectroscopy*

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A discussion of the performance characteristics of an infra-red microspectrometer is given in terms of the cross-sectional area and minimum volume, V, which can be observed with satisfactory signal-to-noise ratio. Methods of increasing the ratio of measured absorption to spectrometer noise are discussed and five ways are enumerated. It is pointed out that for a microscope objective of numerical aperture NA_m associated with a spectrometer of numerical aperture NA_s, the useful magnification is $(NA_m)/(NA_s)$. The design of reflecting-type infra-red microscope objectives having numerical apertures up to 1.5 is described.

A description of an experimental infra-red microspectrometer is given. Use is made of a commercial spectrometer and conversion from a micro to a macro instrument can be made in a few minutes. In the experimental arrangement no changes were introduced that affected the operation of the spectrometer as a macro instrument. Calculations indicate that by making use of other components now available, such as a hotter source and a smaller, more sensitive thermal detector, it will be possible to obtain infra-red spectral measurements of specimens whose linear dimensions approximate those set by diffraction.

Examples of infra-red spectra of crystals, fibers, and tissues of microscopic area are shown. Comparisons of spectral data with those obtained for macro samples are made and an indication of the experimental limitations of the technique is given.

I. INTRODUCTION

ALTHOUGH absorption spectrometry has proved to be a highly useful tool in the identification and, in certain cases, the quantitative estimation of organic chemical compounds,[1] the use of ultraviolet[2] and visible[3] spectrometry with solid samples of microscopic size has been infrequent; and there seem to be even fewer references to the use of microspectroscopy in the infra-red region.[4] In this communication we shall describe and discuss some theoretical and experimental considerations such as minimum sample dimensions and volume which are inherent to the determination of the near infra-red absorption spectra of small amounts of organic substances, and finally present some results obtained with an infra-red microspectrometer now in use in our laboratory.

The infra-red spectra of both saturated and unsaturated organic compounds (except optical enantiomorphs and certain high polymers) are highly characteristic and specific. With the advent of the modern recording infra-red spectrometer,[5] a considerable body of data is now being collected which permits empirical correlation of absorption regions and particular atomic groupings.[6,7] Unfortunately many materials are available only in such small amounts, of the order of milligrams or micrograms, that the usual techniques of infra-red spectrometry are not applicable. In other cases studies of single crystals, which may yield valuable data, have been rare because of the difficulties in obtaining crystals of the proper thickness and large enough size substantially to cover the entrance slit of the spectrometer. The use of the techniques of microscopy for such determinations has long been considered.[8]

The availability of reflecting-type microscope objectives containing no refracting elements[9–12] has made possible the determination of the infra-red spectra of smaller amounts of material than have heretofore been used. Barer, Cole, and Thompson[4] have determined the infra-red spectra of a variety of materials using *aspherical* reflecting microscope objectives. In our laboratory we have devised an instrumental set-up which makes use of *spherical* reflecting microscope objectives† and allows the conversion of a Perkin-Elmer single-beam spectrometer to a *micro*spectrometer in an inexpensive, quick, and convenient manner. We have used this system for the determination of the infra-red spectra of representative organic crystals, fibers, tissue sections, and blood smears.

II. "RESOLVING POWER" TREATED IN TERMS OF VOLUME

A discussion of the ultimate performance characteristics of an infra-red microspectrometer differs from the

* Presented in part at the Buffalo Meeting of the Optical Society of America, October 1949.

[1] (a) H. W. Thompson, Ann. Repts. on Progress Chem. (Chem. Soc. London) **42**, 5 (1945), (b) E. A. Braude, Ann. Repts. on Progress Chem. (Chem. Soc. London) **42**, 105 (1945).

[2] T. Caspersson, J. Roy. Microscop. Soc. [3] **60**, 8 (1940).

[3] E. E. Jelley, Ind. Eng. Chem. Anal. Ed. **13**, 196 (1941).

[4] Barer, Cole, and Thompson, Nature **163**, 198 (1949).

[5] V. Z. Williams, Rev. Sci. Inst. **19**, 135 (1948).

[6] H. W. Thompson, J. Chem. Soc. **1948**, 328.

[7] Barnes, Gore, Stafford, and Williams, Anal. Chem. **20**, 402 (1948).

[8] R. B. Barnes (private communication).

[9] D. D. Maksutov, U.S.S.R. Patent No. 40,859 (December 13, 1932); for references to the Russian work, see E. M. Brumberg, Nature **152**, 357 (1943).

[10] C. R. Burch, Proc. Phys. Soc. (London) **59**, 41 (1947).

[11] Land, Blout, Grey, Flower, Husek, Jones, Matz, and Merrill, Science **109**, 371 (1949).

[12] D. S. Grey, J. Opt. Soc. Am. **39**, 723 (1949).

† The Polaroid-Grey numerical aperture 0.4 objectives have been used to obtain the infra-red spectrum of a single crystal of penicillin (private communication from Dr. R. B. Gore).

usual treatment of microscope performance for two reasons. First, the lower limit of the cross-sectional area which can be studied is in many cases set not by the resolving power of the microscope objective, but by the signal-to-noise ratio‡ on the recorded spectrum. Second, the absorption produced by the specimen may be so weak that a path length through the specimen of several microns is required to give a signal distinguishable above spectrometer noise. Thus, it becomes necessary to treat resolving power not in terms of the smallest *area* which can be observed, but in terms of the *smallest volume V which can be observed with satisfactory signal-to-noise ratio.*

A. General Considerations

To facilitate the study of the manner in which V depends on the numerical aperture of a microscope objective associated with a spectrometer, define an equivalent numerical aperture NA_s of the spectrometer. The equivalent numerical aperture NA_s is the numerical aperture of a collimator with circular aperture which would accept from the entrance slit an amount of radiation equal to that accepted by the collimating mirror and dispersing member of the spectrometer. By assigning the equivalent numerical aperture NA_s to the spectrometer, we substitute for the rectangular aperture of the dispersing member a round aperture which would accept the same amount of radiation. Furthermore it will be assumed for the moment that the transmission of the spectrometer is not affected by point of incidence at the dispersing member.

A specimen is now placed at the entrance slit of the spectrometer model described above. The height of the slit is denoted by h_s and the width by w_s. The signal-equivalent wave-length pass band is a rectangular wave-length pass band of width $\Delta\lambda$ which contains an amount of radiant energy equal to that in the band accepted by the spectrometer, and which is centered on the peak of the spectrometer pass band, λ_0. The amount of radiant flux, E, accepted by the spectrometer is thus equal to the product of slit area, times equivalent numerical aperture squared, times equivalent band width, times the spectral radiance $I(\lambda_0)$ in the wave-length pass band.

$$E = h_s w_s \pi \langle NA_s \rangle^2 I(\lambda_0) \Delta\lambda. \tag{1}$$

Now let E_m be the minimum value of E which will permit satisfactory operation of the spectrometer with the sample in question. The requirement

$$h_s w_s \geqq \frac{E_m}{I(\lambda_0)\Delta\lambda\pi\langle NA_s \rangle^2} \tag{2}$$

must be observed. For a given spectral radiance $I(\lambda_0)$ and band width $\Delta\lambda$, there is thus a minimum permissible value of $h_s w_s$ with the sample in question. The minimum permissible value of the product $h_s w_s \cdot \Delta\lambda$ is generally attained in commercial infra-red spectrometers approximately when the entrance and exit slits are each narrowed down to that width which is equal to the theoretical resolving power of a microscope of numerical aperture NA_s at the wave-length λ_0 in question. In *one* direction, the dimension of slit width, the spectrometer may view a specimen one face of which has a dimension equal to the resolving power of an objective of numerical aperture NA_s, and at the same time the full *spectral* resolving power of the spectrometer is realized. In the other dimension of the sample face, the direction of slit height, the spectrometer views an area many times greater than the resolving power at NA_s.

To permit improvement of the "resolving power" in the dimension of slit height, a consequent loss of incident radiant flux must be compensated by one or more of the following *methods of increasing the signal-to-noise ratio* of the recorded spectrum:

1. Increase the spectral band width $\Delta\lambda$.
2. Increase the spectral radiance $I(\lambda_0)$ of the source.
3. Alter the energy-detecting process of the spectrometer.
4. Pass the radiation through a more favorable path length in the sample.
5. Increase the numerical aperture over which radiation from the sample is received.

The five possibilities enumerated above may be accomplished as follows:

1. The spectral band width may be increased by opening the exit slit independently of the entrance slit of the spectrometer.
2. The source intensity may be increased. A hotter source than conventionally used is possible, but if it is used, the output of visible and near infra-red radiation increases so rapidly that stray light becomes a serious problem. Recently Rupert and Strong[12] have described their work using the carbon arc as a source for infra-red spectroscopy. Using a double monochromator

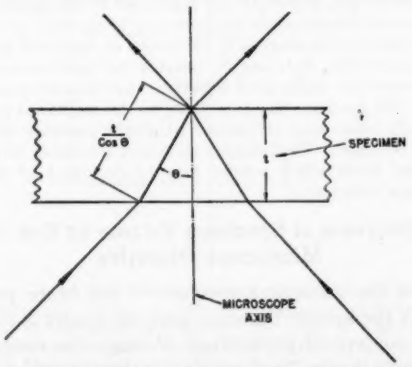


FIG. 1. When a microscope objective is used to image the specimen onto the spectrometer slit, the path length through the sample increases with the obliquity, θ, of rays to the microscope axis. If t is the specimen thickness and θ is the inclination in the specimen of a ray to the optic axis of the microscope, the path length for that ray is $t/(\cos\theta)$.

‡ Signal-to-noise ratio is here used as the ratio of the response in the spectrometer output to any specified absorption band, divided by the noise or random fluctuations in the spectrometer output.

[12] C. S. Rupert and J. Strong, J. Opt. Soc. Am. 39, 1061A (1949).

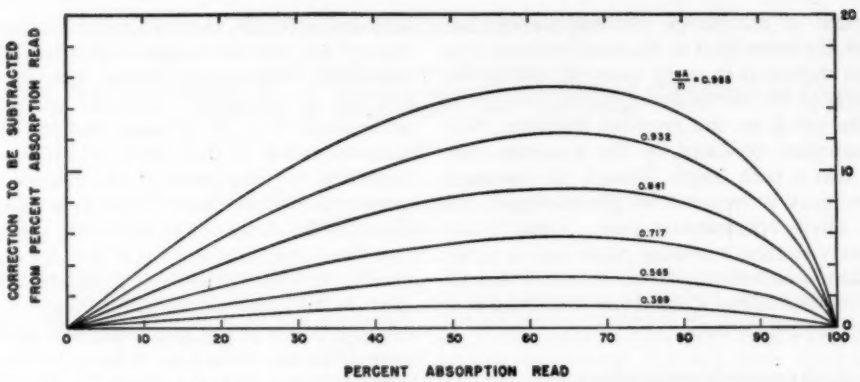


FIG. 2. The percent of incident energy which is absorbed by a sample depends on the absorption coefficient k multiplied by the thickness t along the optic axis of the microscope *and* on the numerical aperture NA_m of the microscope divided by the refractive index n of the specimen. The ratio of absorbed energy to incident energy is $(1-e^{-kt})-\delta$, where δ is the ordinate in the above figure. The subtractive correction δ is plotted against percent absorption read for various values of $(NA_m)/n$.

and correct operating conditions, use of the carbon arc in place of the globar results in a brightness increase of at least five times in the range 6 to 15μ according to these investigations.

3. The signal-to-noise ratio in the recorded spectrum may be improved by using a longer running time together with longer circuit (and detector) time constants, or by using a smaller radiation-detecting element (in accordance with the decreased slit height). There is also, of course, the possibility that intrinsically better sensitive elements may be developed.

4. If the radiation is passed through a longer path in the specimen, a greater amount of energy is absorbed in the absorption bands. Consequently, it may not be necessary to use so large a value of E in order that the absorption bands will show above noise. However, if the thickness of specimen is increased, the effect of the absorption band will increase less fast than does the thickness. If reduction in slit height is compensated by this means, the slit height may not be reduced as much, percentagewise, as the path length is increased; and the specimen volume will become greater rather than less. Therefore, and for other reasons to be given later, it will usually not be desirable to decrease slit height if to do so requires an increase in the thickness of the sample.

5. The amount of radiant flux E received by the spectrometer from a particular area of the specimen may be increased by placing the specimen on the stage of a microscope of numerical aperture NA_m greater than NA_s and by imaging the specimen onto the spectrometer slit. Although it is obvious that the *area of one cross section* of the specimen may be reduced by this method, it does not necessarily follow that the *volume* which the specimen fills may thus be decreased. The following section of this paper treats the conditions under which volume may be decreased by using a microscope objective.

B. Decrease of Specimen Volume by Use of a Microscope Objective

When the numerical aperture of the beam passing through the sample becomes large, no longer is there a simple concept of path length through the sample. If the sample is visualized as being in the shape of a wafer with flat face normal to the microscope axis, it is seen that the path length of a given ray through the specimen is the thickness of the wafer divided by the cosine of the angle of obliquity in passing through the sample (see Fig. 1). For rays at increasingly large numerical aperture, the longer path length gives a greater amount of absorption. This effect must be examined for two properties: the increased absorption may work in our favor by increasing the depth of absorption bands thus permitting a smaller value of E; and the recorded data may lead to erroneous interpretation if it is assumed that the path length for all rays is equal to the path length at small numerical aperture. If the specimen is isotropic, both of these effects are negligible until the numerical aperture of the objective becomes very large.

Consider rays which, within the specimen, are inclined to the optic axis of the microscope objective at angles between and including the values θ and $\theta+d\theta$. The amount of radiation before absorption associated with rays in this range is equal to

$$I(\lambda_0)\Delta\lambda 2An^2\pi \sin\theta \cos\theta d\theta,$$

where n is the refractive index of the specimen, and A is the area of the slit image. The amount of radiation absorbed by the specimen from the radiation thus associated with the obliquity θ is:

$$I(\lambda_0)\Delta\lambda 2An^2\pi \sin\theta \cos\theta[1-\exp(-kt/\cos\theta)]d\theta, \quad (3)$$

where t is the path length through the specimen along the optic axis and k is the absorption coefficient of the specimen. The added absorption arising from obliquity is the difference:

$$I(\lambda_0)\Delta\lambda 2An^2\pi \sin\theta \cos\theta \times\{(1-\exp(-kt/\cos\theta)-[1-\exp(-kt)]\}d\theta. \quad (4)$$

If E_0 is the amount of radiant flux which would be computed as absorbed when the change in path length with θ is neglected, and E_θ is the actual amount absorbed, then the difference $(E_\theta-E_0)$ divided by $E-E_0$ is given by

$$\frac{\int \sin\theta \cos\theta[\exp(-kt)-\exp(-kt\sec\theta)]d\theta}{\exp(-kt)\int \sin\theta \cos\theta d\theta}, \quad (5)$$

where the integration is from $\theta=0$ to $\theta=\sin^{-1}(NA_m/n)$. Expression (5) reduces to

$$\frac{\int \sin\theta\cos\theta[1-\exp(kt-kt\sec\theta)]d\theta}{\int \sin\theta\cos\theta d\theta}. \quad (6)$$

The expression (6) has been evaluated by numerical integration for various values of kt and NA_m/n. The results are plotted in Fig. 2 in the form of a correction to be subtracted from percent absorption read as ordinate *vs.* percent absorption read as abscissa, for various values of (NA_m/n). If the expression (6) is set equal to P, we have

$$P=\frac{E_\theta-E_0}{E-E_0},$$

$$\frac{E_0}{E}=\left(P\frac{E}{E_0}+1-P\right)^{-1}=B,$$

$$E_0=E_\theta B-E_\theta-C,$$

$$E_\theta-E_\theta B=C \quad \text{(ordinate of Fig. 2)},$$

$$\frac{E_\theta}{E}=\frac{E_0}{E}\frac{1}{B}=\frac{1-\exp(-kt)}{B} \quad \text{(abscissa of Fig. 2)}.$$

From Fig. 2 it is apparent that in estimating the gross character of the dependence of the minimum specimen volume V on the numerical aperture NA_m of the microscope, it is safe to neglect the added absorption due to obliquity.

Let h_s^* and w_s^* be the minimum slit dimensions which will give satisfactory signal-to-noise ratio with the specimen at the slit. The same amount of radiant flux may be collected from an area of dimensions $h_s^*(NA_s/NA_m)$ and $w_s^*(NA_s/NA_m)$, and imaged onto the spectrometer slit. (The magnification produced by the microscope must be at least as great as NA_m/NA_s.) Hence, when the small effect of added path length is not considered, we find from Eq. (2) that the minimum cross-sectional area of the specimen taken perpendicular to the microscope axis is

$$\frac{E_m}{I_0(\lambda)\Delta\lambda(NA_m)^2}.$$

Cross-sectional areas distance z above and below the minimum will be

$$\frac{E_m}{I_0(\lambda)\Delta\lambda(NA_m)^2}+2(h_s^*+w_s^*)\frac{NA_s}{NA_m}\left[\tan\left(\sin^{-1}\frac{NA_m}{n}\right)\right]z+\pi\left[\tan\left(\sin^{-1}\frac{NA_m}{n}\right)\right]^2 z^2. \quad (7)$$

If the perpendicular path length through the specimen is t, the minimum volume V is found by integrating (7) with differential dz, between the limits $-t/2$ and $t/2$.

$$V=t\left\{\frac{E_m}{I_0(\lambda)\Delta\lambda\pi(NA_m)^2}+(h_s^*+w_s^*)\frac{NA_s}{NA_m}\left[\tan\left(\sin^{-1}\frac{NA_m}{n}\right)\right]t+\pi\left[\tan\left(\sin^{-1}\frac{NA_m}{n}\right)\right]^2\frac{t^2}{12}\right\}. \quad (8)$$

The first three terms in the series expansion of V are

$$t\left\{\frac{E_m}{I_0(\lambda)\Delta\lambda\pi(NA_m)^2}+(h_s^*+w_s^*)NA_s\frac{t}{n}+\pi(NA_m)^2\frac{t^2}{12n^2}\right\}. \quad (9)$$

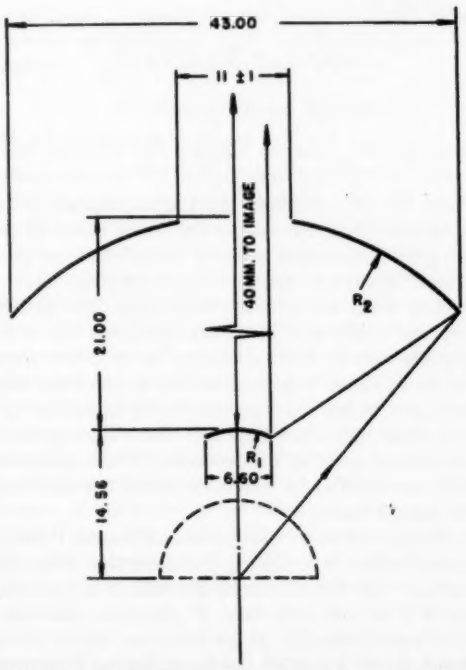


FIG. 3. Convertible infra-red microscope objective. Two spherical mirrors may form a microscope objective suitable for use in infra-red at numerical apertures of the order of 0.65. A single hemisphere of silver chloride or thallium bromide-iodide may be added to the objective (as indicated by dotted lines) to multiply the numerical aperture by the index of the refracting medium. The hemisphere introduces no aberration; this objective is thus convertible for use at numerical aperture 0.63 (no hemisphere), 1.25 (silver chloride hemisphere), or 1.5 (thallium bromide-iodide hemisphere). The numerical aperture cannot, of course, exceed the refractive index of the material in which the specimen is embedded. A meniscus lens of either of these materials or rocksalt may be inserted to give numerical aperture between 0.63 and 0.9 in a "dry" system.

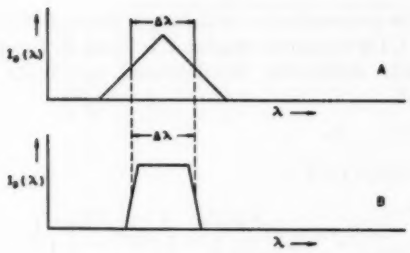


FIG. 4. The energy lost in viewing a very small *area* of the specimen may not be completely compensated by the increased *numerical aperture* over which radiation from the specimen is accepted. A further gain in energy may be realized by increasing the spectral band width passed by the monochromator. This may be done by opening the exit slit independently of the entrance slit (giving the spectral pass band *B*) or by increasing the magnification provided by the microscope and opening *both* slits (giving the spectral pass band *A*).

Hence, from (2) and (9):

$$V \geqq \frac{t}{\pi(NA_m)^2}\left\{h_s^* w_s^* (NA_s)^2 + (h_s^* + w_s^*) NA_s \pi (NA_m)^2 \frac{t}{n} + \frac{\pi^2}{12n^2}(NA_m)^4 t^2 + \cdots\right\}. \quad (10)$$

From Eq. (8), or from the partial expansion (10), it is evident that there is an optimum value of NA_m which gives a minimum value of V, and that one should *not* expect always to reduce V by increasing NA_m.

Further facts are evident from Eqs. (8)–(10): The absorption coefficient, k, of the specimen has a very strong effect on V. The thickness, t, is inversely proportional to k; hence k appears as the minus first, minus second, and minus third powers in the expansion of V. Hence, when Lambert's Law and Beer's Law apply, the the necessary number of molecules of the constituent studied decreases as the concentration of the constituent in the sample increases.

If the spectral band width $\Delta\lambda$ is increased, V may be decreased either by reducing the necessary dimensions h_s^* and w_s^* (see Eq. (2)) or by decreasing the necessary value of t. In the first case, V does not decrease as rapidly, logarithmically, as $\Delta\lambda$ increases; but in the case $h_s^* \gg w_s^*$, $h_s^* \gg (NA/n)^2 t^2$, the logarithm of V decreases almost as rapidly as the logarithm of $\Delta\lambda$ increases. On the other hand, if t is permitted to decrease as $\Delta\lambda$ increases, we find first of all that the logarithm of t may decrease *faster* than the logarithm of $\Delta\lambda$ increases. Furthermore, as previously mentioned, the logarithm of V decreases faster than does the logarithm of t. An increase in $\Delta\lambda$ may be used to decrease V and, unless other circumstances prohibit this course, ***V will be reduced most rapidly if the consequent gain in signal is used as permission to decrease t.***

It is apparent that if sectional prisms are placed over the microscope aperture and over the entrance slit, the slit may be "imaged" onto a square area of the specimen.[14] In this case, the factor $(h_s^* + w_s^*)$ of Eq. (8) may be replaced by $2(h_s^* w_s^*)^{\frac{1}{2}}$. In certain cases, this might represent an appreciable reduction of V, but consideration of this and other such devices is outside the scope of this paper.

Finally, it is seen from Eqs. (8) and (10) that the gain to be realized from associating a microscope with the spectrometer is partly illusory. From ordinary experience one has become accustomed to realizing from a microscope a gain in "resolving power" commensurate with replacing the very small numerical aperture of the bundle of rays which the pupil of the eye will accept, by the very large numerical aperture accepted by a microscope objective. But, in infra-red microspectrometry, there is already an equivalent numerical aperture NA_s of the spectrometer; and for commercial instruments, NA_s is of the order of 0.1.

If the magnification between the specimen and the entrance slit of the spectrometer is equal to $(NA_m)/(NA_s)$, no further increase in magnification gives an increase in any figure of merit derivable from specimen volume, signal-to-noise ratio, and spectral resolving power. When the magnification is equal to or greater than this value, all the light which passes through any given specimen within the specified pass band is imaged onto

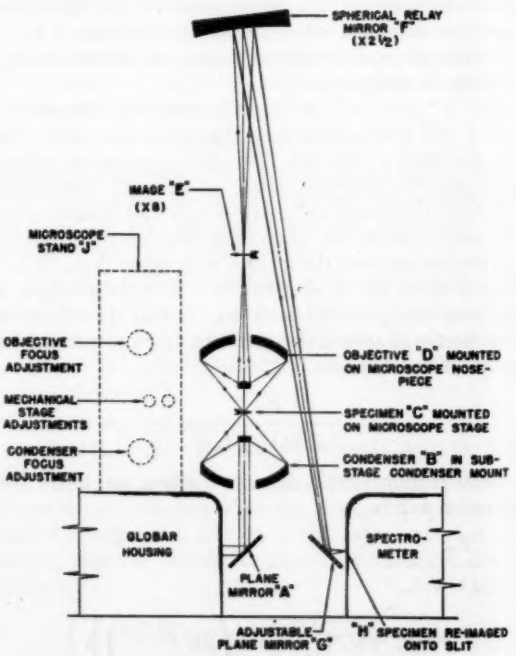


FIG. 5. Diagrammatic drawing of infra-red microspectrometer assembly.

[14] I. S. Bowen, Astrophys. J. 88, 113 (1938).

the detector. The value $(NA_m)/(NA_s)$ may thus be called the useful magnification for an objective of numerical aperture NA_m associated with a spectrometer of numerical aperture NA_s. For a spectrometer with $NA_s=0.1$, and microscope of numerical aperture 1.5, the useful magnification is only 15 times. Although for various conveniences in instrumentation we shall (as in all microscopy) use a magnification a few times greater than the useful magnification, the fundamental gain estimated from using the microscope should be derived from the ratio $(NA_m)/(NA_s)$ and not from the magnification (greater than $(NA_m)/(NA_s)$) used.

III. THE DESIGN OF INFRA-RED MICROSCOPE OBJECTS

Microscope objectives for infra-red may be designed using the Schwarzschild mirrors as the starting point (Fig. 3). The mirrors may be used in spherical form, as shown by Brumberg[9] and his associates for ultraviolet microscopy; non-spherical mirrors may be used as shown by Burch;[10] or the spherical mirrors may be combined with lens elements of silver chloride, rocksalt, etc. after the manner shown by one of us[12] for ultraviolet microscope objectives. The spherical Schwarzschild mirrors are, for infra-red, adequate at numerical apertures up to about 0.65. If these are further combined with a plano-convex lens of silver chloride or thallium bromide-iodide (Fig. 3), there is no difficulty in realizing numerical apertures as large as the refractive indices of infra-red solvents permit.

IV. DETAILS OF ASSOCIATING AN OBJECTIVE WITH A SPECTROMETER

Certain details of operational procedure must be settled before the microscope is associated with the spectrometer.

A. Size of Thermal Detector

It will almost always be true that in microabsorption studies one may use an infra-red-sensitive element much smaller than for macroabsorption studies because the full slit height will not be utilized, or the full aperture of the spectrometer prism will not be filled. In both cases, the used portion of the exit slit may be imaged onto a smaller sensitive area than when the full slit and prism aperture are used. In principle, the signal-to-noise ratio will be inversely proportional to the square root of the sensitive element area provided the area covers the slit image. In practice, the gain realized by this reduction of area is less than as stated above. We have not attempted to capitalize on this possibility, for it involves disrupting the internal mechanism of the instrument.

B. Time for Detection Process

Similarly, if one will use a longer running time to obtain the spectrum, the signal-to-noise ratio may be increased as the square root of the running time. If also the time constant of the bolometer is increased proportionately to the running time, the signal-to-noise ratio may then be increased directly as the running time. We have used longer running time in micro studies than in macro studies, since this can be conveniently accomplished with the Perkin-Elmer spectrometer.

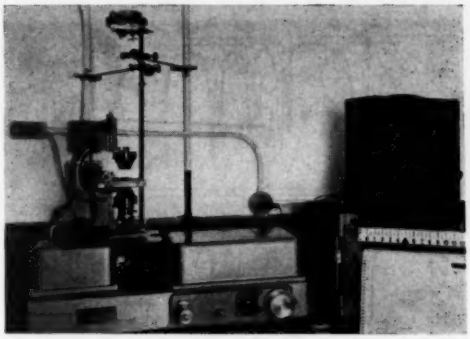


FIG. 6. Photograph of infra-red microspectrometer showing reflecting microscope assembly.

C. Spectral Band Width

The spectral band width $\Delta\lambda$ may be increased by opening the exit slit independently of the entrance slit, or by increasing the magnification (that is, to use a magnification greater than NA_m/NA_s) from specimen to entrance slit and opening *both* spectrometer slits. A result of the latter procedure is that the spectrometer prism is not filled with light. A minor gain is realized from this fact. The transmission of the spectrometer aperture is not, as heretofore assumed, independent of the part of the dispersing prism utilized; for example, the short path through the apex of the prism gives less absorption than the path through the base. Also, a part of the dispersing prism is shadowed by an image of the sensitive element support and housing. In choosing the part of the prism to be used, one must not fail to consider the very appreciable diffraction at the slits. One must not confuse the diffracted path of infra-red radiation with the much less diffracted visible light which one observes in aligning the instrument. For similar reasons, one should be sure that the portion of the exit slit which is used is located midway along the length of the slit. The center of the slit is, for many reasons, more effective in producing signal than are the ends of the slit. Thus in practice, signal is not lost quite in proportion to the amount by which the used length of the slit is reduced.

Essentially the same relation between band width, sensitivity, and V is obviously obtained by either method of varying band width, though the exact shape of the spectral pass band differs, as shown in Fig. 4, depending on which expedient is used. In the former

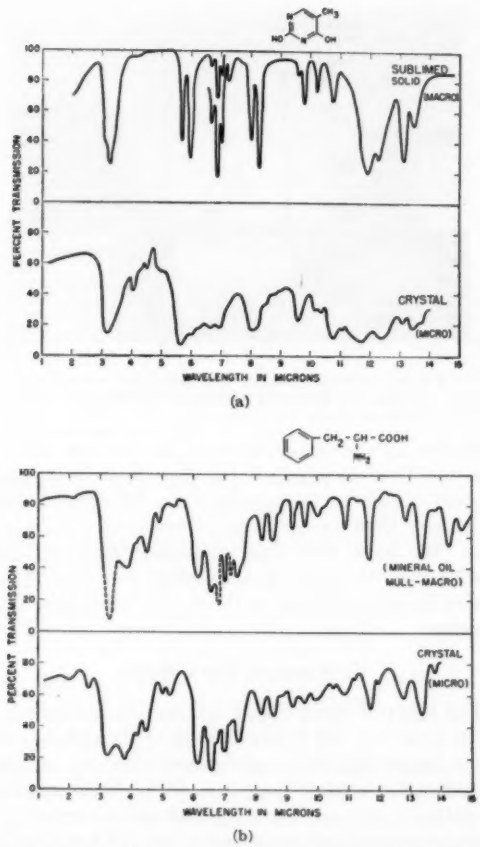


FIG. 7. Infra-red spectra of two organic compounds ((a) thymine and (b) *d,l*-β-phenylalanine) in crystalline state showing comparison of results obtained by micro and macro techniques. Dashed lines indicate places where absorption is caused by mineral oil.

case, the spectral band pass is triangular; in the latter case, it is rectangular. If the band width is increased by opening both slits, it must be recognized that different areas of different size at the specimen are being measured as the slit width is changed, or provision for magnification variable as a function of slit size must be made.

D. Minimum Linear Dimension as Established by Diffraction Theory

Finally, no matter how the performance characteristics of the radiation-detecting components may be altered, it should be remembered that the specimen must have a cross section not smaller than diffraction theory permits. No linear dimension of this cross section may be smaller than about $(0.6\lambda)/(NA_m)$. Although excess magnification may be used in imaging the specimen onto the spectrometer slit, one should not be misled by the high resolving power exhibited in *visible* light. Also, the value of $(0.6\lambda)/(NA_m)$ changes appreciably, druing the course of determing an infra-red spectrum, as λ is varied.

Thus, if it is assumed that our ultimate infra-red microscope will have a numerical aperture of 1.5 and it will be desirable to use the instrument to wave lengths as long as 15μ, *then at least two linear dimensions of an object whose spectrum is to be obtained must be not less than 6μ.* The minimum value of the third dimension of the object is the sample thickness, t, which is determined by absorption coefficients and signal-to-noise ratio of the spectrometer.§

V. EXPERIMENTAL

A. Instrument

The microspectroscopic arrangement in use is shown in the drawing in Fig. 5 and the photograph in Fig. 6. The entire device, including the necessary mirrors and microscope, is bolted to the base of the spectrometer and may be removed in one unit. This arrangement is convenient, since it allows one to alter the spectrometer from a macro to a micro instrument in only a few minutes' time. Radiation from the globar source is reflected by a plane mirror (A) placed at 45° to the exit window of the collimating system. This light is then focused by the reflecting condenser (B) onto the object (C). An approximately 8-times magnified image of the object is then focused by the reflecting objective (D) at (E); and this image is remagnified 2½ times by the spherical mirror (F), which is used slightly off-axis. The resulting image (20 times) lies in the plane of the entrance slit of the spectrometer after reflection by a second plane mirror (H), which is adjustable about two axes.

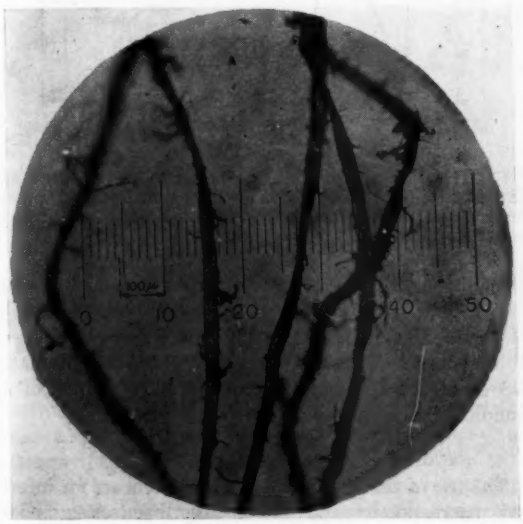
The microscope objectives used have a numerical aperture of 0.40, consist of two spherical reflecting surfaces, and were originally made for ultraviolet work.[11] A new pair of objectives having a numerical aperture of 0.63 has been designed by one of us and is now being constructed by Bausch and Lomb Optical Company (Fig. 3). Since the amount of light collected varies as the square of the numerical aperture, it is expected that these new objectives will substantially improve the obtainable performance. As mentioned previously, the use of a silver chloride or thallium bromide-iodide hemisphere in conjunction with such objectives should make it possible to obtain even higher numerical apertures and still maintain high transparency over the 2 to 20μ range. We find it convenient to mount our specimens on a thin (0.030–0.060 in.) silver chloride disk, which in turn fits into a plastic slide which is the

§ The wide cone of rays at large numerical aperture permits the 6μ linear dimension to be realized in only one cross-sectional plane of the specimen. In order that 6μ is a fair estimate of specimen size, it is necessary that the specimen be thin (less than 10μ thick). This may require a further increase in signal-to-noise ratio, which could perhaps be most conveniently achieved if absorption is measured at only a few wave-lengths. A long time could be allowed to evaluate absorption at each of these few wave-lengths. (See Sec. IIA, Item 3.)

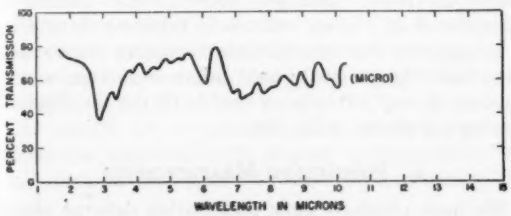
size of an ordinary microscope slide. It is then possible to use the usual x- and y-axis locating mechanism on the stage of the microscope stand. Disks have proved to be particularly satisfactory, since they can be easily rotated in the plastic slide to align small specimens with the spectrometer slit.

The area to be measured is selected using light from the globar in a darkened room. A metal plate held in the sample slide holder of the spectrometer, painted a diffusing white and containing a slit in it slightly larger in both dimensions than the entrance slit of the spectrometer, is a convenient aid in choosing the area to be measured. Some reduction in scattered light may be obtained by placing a slit directly in front of the globar.

In this experimental set-up we have used entrance slit widths varying from 0.050 mm to 1.00 mm. Such slit widths are approximately twice our normal operating slit widths for macro infra-red spectroscopy. We have increased the signal-to-noise ratio by increasing threefold the time necessary to record a spectrum (forty minutes for the region 2 to 15μ). The Perkin-Elmer spectrometer is equipped with a Baird Associates bolometer[15] which uses radiation chopped at 10 c.p.s.



(a)



(b)

FIG. 8. (a) Photomicrograph of ramie fibers. (b) Infra-red spectrum of single fiber of ramie.

[15] R. Clark Jones, J. Opt. Soc. Am. 39, 344 (1949).

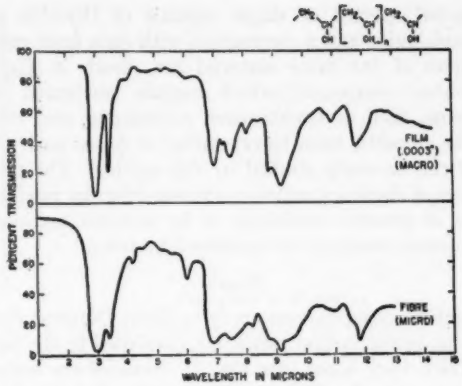


FIG. 9. Infra-red spectrum of polyvinyl alcohol film compared with that obtained from a single fiber.

B. Choice of Specimen. Crystals

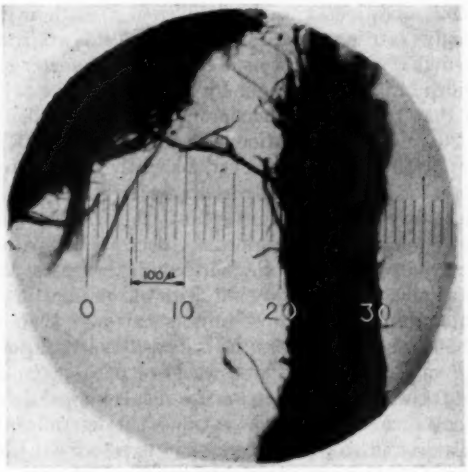
From preliminary work it has become apparent that several conditions must be fulfilled if a single crystal is to give satisfactory data. As to size, if it is arbitrarily stated that the entrance slit of the spectrometer shall be filled in both dimensions by the image of the object, and that 1 mm is the maximum slit width used, then for 20 times magnification, the minimum object size must be 50 by 600μ. However, a slightly shorter object whose image is vertically centered on the slit gives satisfactory signal-to-noise ratio if the width requirement is met. The signal does not decrease, logarithmically, quite as fast as the slit length decreases, logarithmically. It is not possible with our present instrumental components to use objects narrower than 50μ without significant loss in signal-to-noise ratio at long wave-lengths. Because we have fixed magnification, the width of the specimen which is viewed defines the entrance slit width. The entrance slit determines the spectral band width; a decrease in entrance slit width causes a decrease in spectral band width. The signal is thus proportional to the square of the object width, and signal decreases logarithmically *twice* as fast as does object width. It may be pointed out that the variation of slit width with wave-length (to maintain signal-to-noise ratio) causes a proportional variation with wave-length in the specimen width viewed.

The above linear dimensions are possible of reasonably facile attainment with crystals of most organic compounds, but two additional requirements must be met if optimum results are to be obtained. These are related to thickness and freedom from scatter. Those materials which crystallize as plates have been found to be useful generally since they are usually thin (1 to 100μ), whereas those which crystallize as prisms tend to be too thick or scatter too much light. Other unsatisfactory types of crystals are those in which there are large crystals upon which smaller crystals have formed on the surface, thus scattering and losing light. The

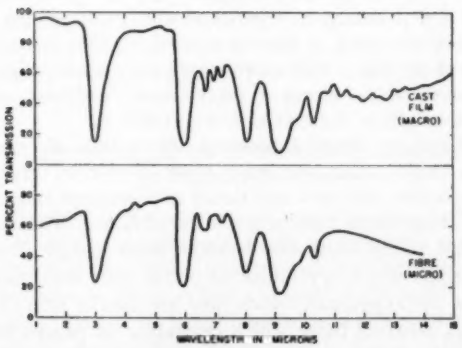
infra-red spectra of single crystals of thymine and phenylalanine and a comparison with data from macro samples of the same material are shown in Fig. 7. Chemical compounds which contain condensed ring systems, such as naphthalene, anthracene, and many of the steroids, seem to crystallize in forms such that they can be easily studied by this method. The possibilities of obtaining suitable crystals either by modifications of growth conditions or by suitable sectioning techniques remain to be examined in detail.

Fibers

Similar considerations apply to fibers. Natural fibers such as ramie, cotton, wool, etc., are used in the form in which they occur (see Fig. 8). Some of the surface scattering can be diminished by coating lightly with a suitable liquid such as a hydrocarbon or perfluorinated oil. We have also had some success in sectioning fibers of high polymers by embedding in wax, cutting with a microtome, and then removing the wax. The polyvinyl alcohol spectrum shown in Fig. 9 was obtained from a drawn fiber sectioned in this manner. When the width of the fiber is sufficiently small (below 2μ), we have been able to use bundles of fibers and obtain useful data (see Fig. 10, a photomicrograph and spectrum of a bundle of nucleohistone fibers).

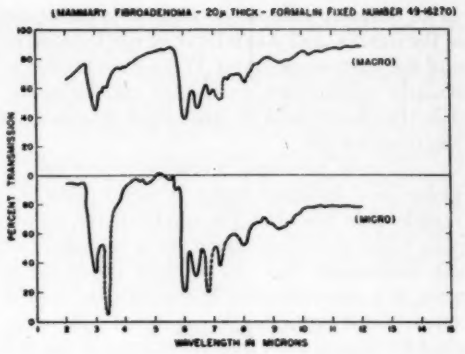


(a)

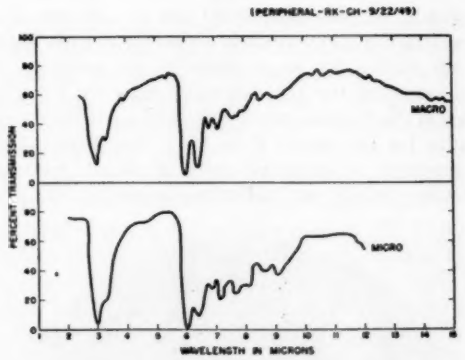


(b)

FIG. 10. (a) Photomicrograph of bundle of nucleohistone fibers. Single fibers have diameters $<5\mu$. (b) Infra-red spectrum of a bundle of nucleohistone fibers (micro) compared with that obtained from a film of nucleohistone.



(a)



(b)

FIG. 11. (a) Comparison of infra-red spectra obtained with same tissue section using macro and micro techniques. (b) Similar data for a peripheral blood smear. Broken lines indicate region of numeral oil absorption.

Tissue

The use of tissue sections and blood smears for infra-red macrospectroscopy has already been described.[16] We have obtained data on such samples, using the micro technique described above. Since the mean diameter of an average cell can be taken as about 10μ, it is apparent that considerable averaging must result even with the presently used micro technique, where between 50 and 500 cells are used to fill the slit. Typical spectra are shown in Fig. 11.

C. Polarization Measurements

We have obtained some polarization data on single crystals using a silver chloride sheet-type polarizer[17, 18]

[16] E. R. Blout and R. C. Mellors, Science **110**, 137 (1949).
[17] N. Wright, J. Opt. Soc. Am. **38**, 69 (1948).
[18] R. Newman and R. S. Halford, Rev. Sci. Inst. **19**, 270 (1948).

placed just below the condenser. A sufficiently high degree of polarization can be obtained by this technique to reveal significant differences in the intensities of the absorption bands of many crystals. The polarizer, however, attenuates the signal strength almost three-fold, so that it is often necessary to use slit widths greater than the slit widths which give good resolution of the absorption bands of certain organic materials.

VI. DISCUSSION

The use of a reflecting microscope in conjunction with an infra-red spectrometer offers the advantage of decreasing the sample size below that possible with

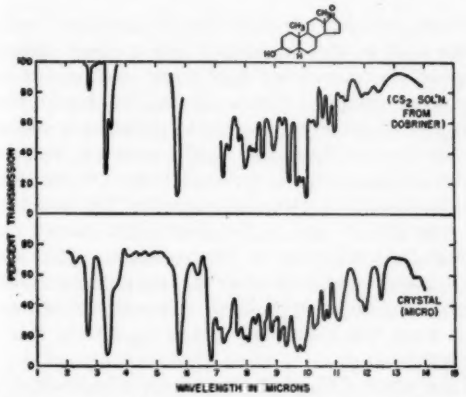


FIG. 12. Comparison of infra-red data obtained from a single crystal of a male hormone, androsterone, with that obtained from a carbon disulfide solution of the same material. The latter data is from K. Dobriner *et al.*, J. Biol. Chem. 172, 297 (1948).

macro techniques. In many cases the absorption bands are so wide that the loss of spectral resolving power does not result in impairment of data. A good example of this is shown in Fig. 12, where comparison is made between the spectrum obtained with a single crystal of androsterone by the micro technique and that obtained from a solution by macro techniques. From such data, observed spectral resolution has been plotted as a function of wave-length (Fig. 13) for the present experimental arrangement. These resolutions represent the best results so far obtained, and for the reasons outlined in Section VI, many materials will give less satisfactory data. It does seem likely that this type of instrument will prove useful in work where materials possessing the necessary physical structure are obtainable.

It should be emphasized that spectral resolving power has been lost in the present instrumental set-up only because no alterations have been made on the

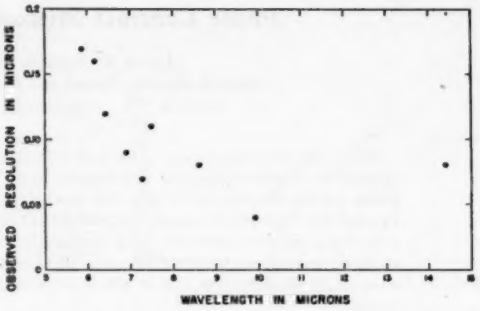


FIG. 13. Points showing wave-lengths and separation in doublets in experimentally observed spectra of micro samples with present instrument.

spectrometer which preclude its use as a macro instrument. Finally, it may be indicated that the sample size limitation as set by diffraction theory (Section V, D) does seem possible of attainment with full *spectral* resolving power of the spectrometer dispersing element through the construction of an apparatus so designed as to give optimum performance with micro samples. This ideal infra-red microspectrometer would use a very hot source such as a carbon arc[13] or zirconium arc; it would use microscope objectives of numerical aperture greater than 1; it would use a heat detector with a high "factor of merit,"[15] and probably use one of small area with a long time constant to gain still more in signal-to-noise ratio. We have made preliminary calculations of the performance of such an instrument using reasonable values for the components, even though no such instrument exists to our knowledge at present. These calculations indicate that the diffraction limit of 6μ linear dimension (at a wave-length of 10μ) will be the limiting factor. This is an interesting figure to the biologist, since many cells have linear dimensions greater than 10μ. Thus it may prove possible to determine the infra-red spectra of single cells or even portions of cells.

ACKNOWLEDGMENT

The authors would like to acknowledge the generosity of the following people in supplying materials used in this investigation: Dr. F. H. Forziati of the National Bureau of Standards for the ramie fibers, Dr. P. M. Doty of Harvard University for the nucleohistone fibers, Dr. R. C. Mellors of the Sloan-Kettering Institute for the tissue section, Dr. M. Pechet of The Children's Medical Center for the blood smears. We wish particularly to thank Miss Adelaide Sutton for valuable technical assistance, and Mrs. J. D. Sykes for the photo-micrographs.

Phase Contrast Microscopy for Opaque Specimens*

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(Received February 24, 1950)

Phase contrast microscopy is by now a familiar subject in the field of transmitted light microscopy. Its application in reflected light is more recent. Early systems for reflected light phase contrast either located the phase annulus directly in the objective focal plane, or else compromised on this desired location and placed it beyond the vertical illuminator, somewhere between aperture and field planes in the microscope. This paper describes a different approach to the problem, resulting in an instrument design which obviates the elaborate equipments required by the above-mentioned systems, requires only one phase annulus and one illuminating annulus for all objectives, and in which the phase annulus is located in the ideal aperture plane of the microscope.

TO convert a normal microscope to a phase contrast microscope, two alterations are required: the illuminating pupil must be restricted, generally in the form of an annulus for reasons of symmetry and sensitivity, and the image of this annulus must be superimposed on a phase-altering annulus located in the pupil of the image-forming system. The phase altering annulus must also be semitransparent in order to render the diffracted and undiffracted portions of the light energy roughly equal, thereby achieving maximum contrast in the image which is formed by mutual interference of these two portions of the light energy. Our problem was to apply such a phase contrast system to a microscope designed for opaque specimens. Such microscopes have come to be known as metallographic or metallurgical microscopes as their major application has been in the field of metallurgy, but they have been used in other fields as well.

Prior systems for accomplishing phase contrast on opaque specimens either located the phase annulus directly in the objective focal plane [1,2] or else compromised on this desired location,[3–5] placing it beyond the vertical illuminator, somewhere between aperture and field planes in the microscope. There are practical objections to both of these systems. Locating the phase annulus in the objective gives rise to flares due to reflections from the annulus. In existant microscopes, locating it between the vertical illuminator and the eyepiece results in uneven illumination and also causes the optics to perform less efficiently due to the radical pupil shift for off-axis pencils. Taylor[5] describes a system to overcome these troubles, in which each objective carries its own vertical illuminator and phase annulus, the design being compressed to the point that the pupil location is not far from normal.

Our first attempt to apply phase contrast to an opaque specimen probably paralleled that of every other worker in the field, in that we simply took a phase objective designed for transmitted light work, and used it on a microscope equipped with a vertical illuminator, modifying the illumination system to deliver the required annular beam of light. The results were fair, but never wholly satisfactory, due principally to reflected flares from the metallic-coated phase annulus. The system also was wasteful of light as the direct light passed twice through the phase pattern. Precise leveling of the specimen was also required in order that the phase annulus be imaged directly back on itself, and many metal specimens were not sufficiently plano to satisfy this requirement.

If the image of the clear annulus could be relayed into an accessible plane beyond the vertical illuminator, the necessity of using a compromise position for the phase pattern, or of accepting the above-mentioned defects where the annulus is located directly in the objective, would be obviated. Early experiments showed that a relay system would indeed do not only this but in addition would materially simplify the phase equipment, since a single illuminating annulus and a single phase annulus sufficed for the whole series of standard objectives. This was due to the fact that in vertical illumination each objective acted as its own condenser, and the size of the aperture stop image remained substantially the same regardless of objective focal length.

The success of this initial step caused the project to graduate at this point from a research to an engineering status. The problem became that of designing a basic instrument capable of application on any of three current models of inverted "metallographic microscopes." Optically two of these instruments were identical; hence the problem was essentially that of adapting the design to two existant microscopes.

Early considerations were given to designing a straight-tube relay system which could be used in either the visual or camera eyepiece adapter tubes of these photomicrographic instruments, but as the design progressed it became apparent that a more convenient system could be worked out, using the camera eyepiece adapter tube only. The unit then became *L*-shaped and

* Presented at the New York meeting of the Optical Society of America, March 1950.

[1] Cuckow, Nature **159**, 639 (1947).

[2] Cuckow, "The Phase Contrast Incident Light Microscope," Iron and Steel Inst. J. **161**, 1 (1949).

[3] Jupnik, Osterberg, and Pride, J. Opt. Soc. Am. **36**, 710A (1946).

[4] Jupnik, Osterberg, and Pride, J. Opt. Soc. Am. **38**, 338 (1948).

[5] Taylor, J. Roy. Microscop. Soc. **69**, 49 (1949).

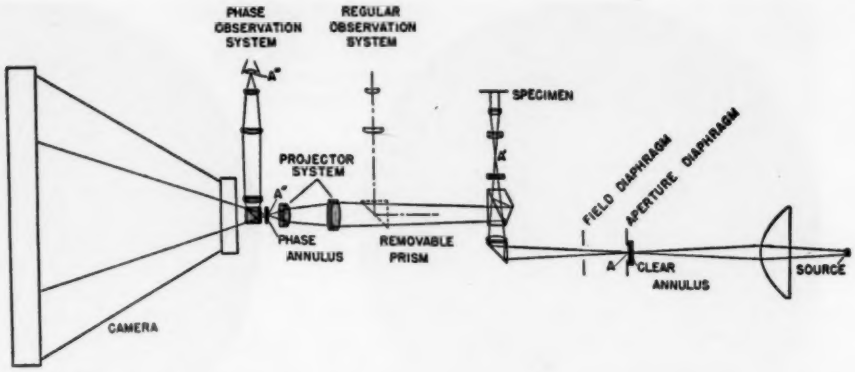


FIG. 1. The phase contrast accessories in place on a reflected-light microscope. The accessories are shaded to differentiate them from the microscope proper.

employed a beam-divider to transmit part of the energy to the camera and reflect the remainder to the new visual eyepiece. Figure 1 shows this system in place on an inverted metallographic microscope. The shaded parts represent the new phase accessories, the rest being the microscope proper. Figure 2 is a photograph of the combination. In Fig. 1 the clear annulus is located at the point A close to the aperture diaphragm, and is imaged by the condenser system into the rear focal plane A' of the objective. The specimen, being plano, returns the rays along their incident paths and an image of A' is formed at unit magnification back on itself. The 4-element lens system in the phase accessory forms an image of A' in the plane of the phase annulus at A''. The beam-dividing cube then transmits a phase contrast image directly to the photographic plate and reflects an image into the simple telescope for visual phase observation. The eyepiece in this viewing system may be any microscope eyepiece which the observer desires. The final pupil image is formed at the eyepoint A''' as indicated in Fig. 1.

One basic feature of the design was the problem of correcting the pupil at A'' for freedom from spherical and chromatic aberration, at the same time maintaining the field corrections of a normal photomicrographic ocular. None of the ordinary oculars were well enough corrected for pupil aberrations, and as a result gave a colored haze to the field at some little distance off axis. Most of the pupil aberration in these oculars arose from the field lens, and early efforts were directed toward a 3-element design in which the field lens was a doublet and the "eye lens" a singlet. A system corrected in itself for field and pupil aberrations was found possible, but sufficient pupil overcorrection to compensate the undercorrection (primarily chromatic) of the condenser system was not achieved. The addition of another lens element in the "eye lens" produced the desired over-all correction, giving satisfactory performance for both field and pupil corrections. This 4-element design had a magnification of 5× for a 10-in. camera bellows draw.

On a normal metallographic microscope the objectives are parfocal, hence the back focal planes do not fall in the same position A' (Fig. 1) for the different focal length objectives. To compensate for this variation it is necessary to provide a focusing means for the projector system in order to produce a sharp pupil image at A'' for all objective powers. This results in about a 5 percent pupil size change in going from highest to lowest power (1.8 mm to 32 mm) objectives. This is taken into account in designing the phase disk slightly thicker than the image of the clear annulus, so that the phase annulus always overlaps the clear annulus image.

The pupil alignment is carried out by means of a small auxiliary telescope, as in the transmitted light system. The eyepiece is removed and the telescope inserted in its place. The projector system is focused to form a sharp image of the clear annulus in the plane of the phase annulus, and the latter is then centered to the former by means of two centering keys. Replacement of the telescope by an eyepiece completes the adjustment, and phase observation is then accomplished.

Having achieved a system for opaque phase contrast, the next question which arises is what the phase image adds to the already available knowledge of the specimen, gathered from brightfield, darkfield, polarized light, oblique, or conical illumination. The answer appears to

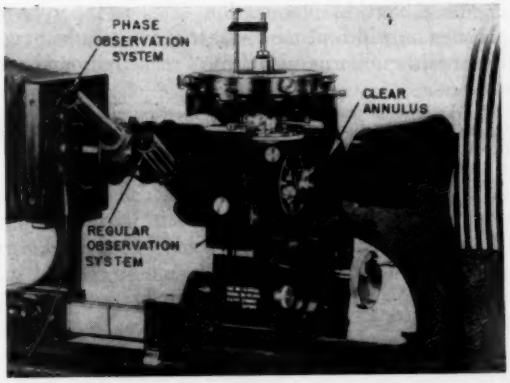
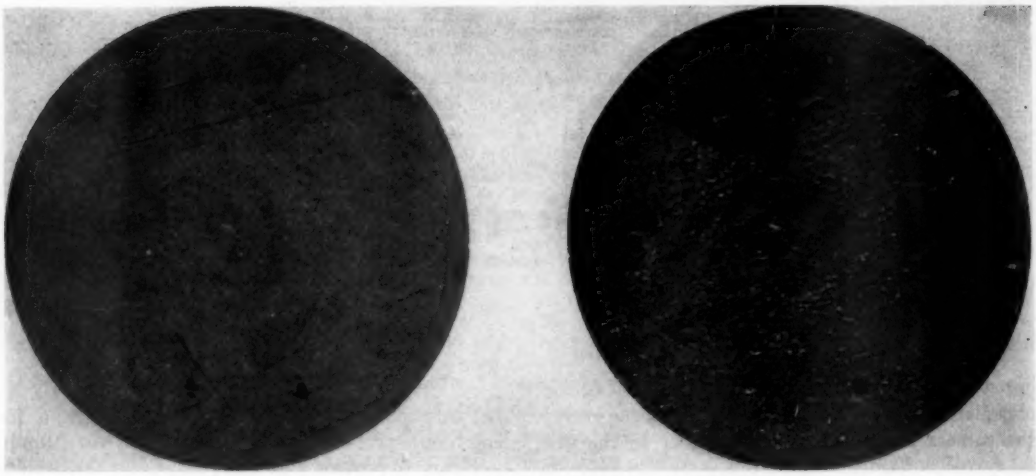


FIG. 2. A photograph of the combination as shown schematically in Fig. 1.



(a) (b)

FIG. 3. A bright field (a) *vs.* phase contrast (b) comparison photomicrograph. The specimen is unetched tin.

be an increased knowledge of surface configuration, and a new means of differentiating some of the true opaques by virture of their characteristic phase shifts at normal incidence.[6] The phase contrast image is similar in its origin to the interference microscope image[7] and a similarity in the actual images is to be expected, although the images will not be identical. The phase image is complicated by the fact that factors other than surface elevation and phase shift at reflection enter into the picture. In a sense the final image must be assumed to include not only these phase effects, but brightfield effects due to different coefficients of reflection, and darkfield and conical illumination effects due to scattering on a comparatively gross scale. The relative contribution of these effects can be judged, of course, by comparing the phase contrast image with the brightfield, darkfield, and conical illumination images.

Figure 3 is a photomicrograph of an unetched tin specimen, taken in both phase contrast and in normal brightfield vertical illumination. This is the type of specimen on which phase contrast shows to advantage. It is of fairly uniform coefficient of reflectivity and contains no rough areas to bring out contrast in the brightfield image. The phase contrast picture shows the surface configuration which is invisible in the brightfield picture. On the other hand it is only fair to say that most specimens etched to bring out contrast in brightfield show to less advantage in phase contrast. Etching of opaques is somewhat analogous to differential staining of transparent structures. The transmitted light phase contrast microscope has found its principal field of usefulness on materials which for one reason or another cannot be stained and it may well be that the opaque phase microscope will find an analogous application on materials which either cannot be etched successfully or on which the etching is adjudged to introduce artifacts. There is moreover the possibility that as more applications are found for opaque phase microscopy special etching techniques or other surface treatment may be applied to bring out the phase image in place of the present techniques designed to bring out the normal brightfield image.

Transparent materials can be examined with the opaque phase set-up simply by backing them with a mirror. The light then passes through the material twice, thereby doubling the phase retardation effects, so that increased sensitivity over the normal transmitted light system is possible.

[6] F. A. Jenkins and H. E. White, *Fundamentals of Physical Optics* (McGraw-Hill Book Company, Inc., New York, 1937), pp. 401–403.

[7] Industrial Diamond Review **6**, 276 (1946).

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Microspectroscopy*

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Microspectroscopy involves the combination of microscope and spectroscope optics for the purpose of investigating the spectra of small samples. The general methods of microspectroscopy are described as applied to absorption measurements in the ultraviolet, visible, and infra-red regions, emission spectroscopy, and other applications. Recently developed components and systems for microspectroscopy are emphasized, and some of the important requirements for their effective design and use are considered briefly.

INTRODUCTION

THE term "microspectroscopy" may be used to refer to any combination of microscope and spectroscope optics for the purpose of studying the spectra of small specimens or portions of larger ones. The types of specimens that may be observed include single crystals, minute amounts of liquids or solutions, thin solid films, biological cells and tissues, and small sources or small areas of sources. As compared with corresponding macromethods, the weight of material required for obtaining microabsorption, emission, or fluorescent spectra may be reduced by factors up to 10^{-3} or even 10^{-4}, a fact of considerable importance in microanalytical procedures. Infra-red and ultraviolet microabsorption spectroscopy of small crystals or solid films (especially when combined with polarization and low temperature techniques) promise to be useful in studies of molecular structure and in investigations of the solid state. Ultraviolet microabsorption spectroscopy has made possible the determination of the distribution of important biochemical substances within biological cells or tissue in various states of normal development or of pathological disfunction; such applications have been of particular interest to biologists and medical investigators.

In principle, microspectroscopy is applicable to absorption spectroscopy of the infra-red, visible, ultraviolet and vacuum ultraviolet regions, and to Raman, fluorescence, and emission spectroscopy. In practice, the technique was long limited to the visual observation of spectra with conventional microscopes and eyepiece spectroscopes. More than a decade ago, Caspersson[1] added a sector photometer and photo-cell to a Zeiss quartz microscope illuminated by a monochromator, and demonstrated that it was possible to determine quantitative ultraviolet absorption spectra of small portions of biological cells and tissues. The measurements were restricted to a few wave-lengths corresponding to the emission lines of arc or spark sources, but the information gained was of sufficient biological significance to stimulate interest in the technique. There was little improvement in Caspersson's original methods[1] until recent years, when rapid advances have been made as a result of the design of microscope optics of the reflection type, achromatic over wide ranges of the spectrum, and as a consequence of continued improvement in radiation-detecting devices, monochromators, light sources, and other components.

The present paper is a general survey of the methods of microspectroscopy with emphasis upon some of the newer systems and their applications. Investigations involving ultraviolet microscopy and microabsorption spectroscopy have been reviewed up to 1948 by Scott and Sinsheimer[2] and will be referred to only briefly, although it should be remembered that the current developments derive in considerable part from this earlier work. Several of the systems and components discussed herein will be described in detail in subsequent publications.

GENERAL CONSIDERATIONS

Three methods for combining microscope and spectroscope optics will be considered. In Method I, the desired portion of the microscope image is focused on the slit of a spectroscope or spectrograph and the resulting spectrum is observed visually or photographed. This method requires that the microscope objective be well achromatized and corrected for other aberrations throughout the spectral region to be observed. In Method II, a monochromator is used to illuminate the microscope and the desired portion of the microscope image is focused upon a radiation detector in such a manner as to permit quantitative transmission measurements to be made. While applicable in principle to infra-red, visible, and ultraviolet microabsorption spectrophotometry, this method has been used primarily for measurements in the ultraviolet. In Method III, the output of the microscope is fed into a monochromator and thence into a radiation detector. This method has been used for microabsorption spectroscopy in the infra-red, but has been avoided in ultraviolet studies because of the possibility that prolonged irradia-

* Based upon an invited paper presented at the Buffalo meeting of the Optical Society of America, October, 1949. Certain of the investigations described were supported by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council.

[1] T. Caspersson, Skand. Arch. f. Physiol., Supplement 73 (1936).

[2] J. F. Scott and R. L. Sinsheimer, "Ultraviolet microscopy and microspectroscopy," in *Medical Physics* (Year Book Publishers, Inc., Chicago, in press), Vol. 2.

TABLE I. Examples of special objectives suitable for microspectroscopy.

Designer	E.F.L. mm	N.A.	Remarks
I. Quartz monochromats (usable from 2300 to 3500A with maximum Δλ of 2 to 10A)			
Kohler	16.0	0.20	Dry
	6.0	0.35	Dry
	2.5	0.85	Immersion
	1.7	1.25	Immersion
II. Quartz—fluorite achromats			
Johnson	25.0	0.2	Usable 2200–5500A, Δλ approximately same
Foster	2.5	0.85	Immersion, Δλ *ca.* 100A, usable 2300–3500A
III. Two-mirror, dry-type reflection objectives (no chromatic aberration; usable at least 2000A to 15μ, essentially unlimited Δλ)			
Burch	3.0	0.65	One aspherical surface; O.R.=0.2
Grey	2.4	0.4	O.R.=0.4
Kavanaugh	3.2	0.56	O.R.=0.5
IV. Two-mirror, immersion-type reflection objectives (characteristics as for Type III except for lower coverslip aberrations and limitation of transmission by front dielectric element).			
Burch	3.0	0.98	O.R.<0.2
Kavanaugh	2.5	0.80	O.R.<0.5
	2.8	0.85	
V. Two-mirror systems with quartz and fluorite refracting elements (transmission range limited to that of quartz; Δλ essentially unlimited in this range).			
Grey	2.8	0.72	O.R. *ca.* 0.3

Notes.—(1) The immersion fluids usually used are glycerine or glycerine and water. (2) Slides and cover-slips of quartz or high silica glass may be used in the visible and ultraviolet (to 2200 or 2000A). (3) Rocksalt slides are ordinarily employed in the infra-red. (4) The quartz monochromats described are those formerly manufactured by Zeiss Optical Works. A few similar objectives have been made by A. and J. Beck, Ltd., and by Bausch and Lomb Optical Company. Objectives of the same type but of new design are currently produced by Cooke, Troughton, and Simms, Ltd. (5) O.R. refers to approximate linear obscuring ratio.

tion of the specimen with the full ultraviolet spectrum might lead to photo-decomposition.

Conventional glass microscope optics generally have sufficient transmission for use in the spectral range 3400 to 10,000A, but unless a well-corrected apochromat is employed it may be impracticable to cover a range greater than 2000 to 3000A without refocusing. For shorter wave-lengths in the ultraviolet or longer wave-lengths in the infra-red, other types of optics must be employed. Table I lists some representative objectives for extension of microspectroscopy beyond the visible range.

Quartz microscope objectives of the type originally designed by Kohler[3] have been employed extensively for ultraviolet microabsorption spectroscopy by Method II. These are monochromats, designed and corrected for use at one wave-length (usually 2750A), but they may be used over a considerable wave-length range (about 2300–3500A) if refocused for each wave-length. The half-intensity spectral band width, Δλ, must be kept within about 2 to 10A, depending upon the objective and conditions of use, for satisfactory image quality, since the chromatic aberrations are marked.

Quartz-fluorite objectives have been designed by Johnson[4] and Foster,[5] among others. At high N.A. (e.g., 0.85) it is not feasible to correct such objectives for Δλ greater than about 100A, but even this gain in acceptable band width permits less critical focusing than with monochromats and simplifies the problem of monochromatic illumination. Such objectives are far easier to use for Method II than quartz monochromats, and even those of relatively high aperture may be employed for Method I to cover restricted spectral ranges.

The most promising advances in extending the spectral range of transmission and achromatization of microscope optics have involved mirror systems. These include single spherical or aspherical mirrors, with or without refracting correction plates or auxiliary lenses, two-mirror spherical systems (Brumberg,[6] Grey,[7] Kavanaugh,[8] Leeds[9]), two-mirror aspherical systems (Burch[10]), and two-mirror spherical systems combined with refracting elements (Grey[7]). The introduction of refracting elements has usually been for the purpose of correcting residual errors of the mirrors, but Grey has adopted the novel approach of dividing the power between the mirrors and lenses, thus achieving greater flexibility in design. All-mirror systems are usable throughout the infra-red, visible and ultraviolet and, if figured with sufficiently close tolerances, even in the vacuum ultraviolet. Most of the combined refraction and reflection systems involve the use of elements of

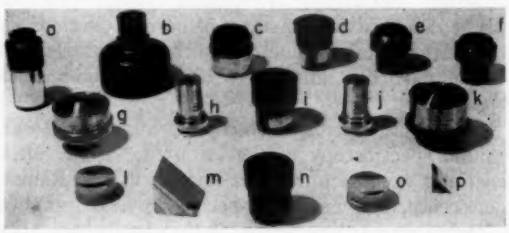

FIG. 1. Recently designed elements for microspectroscopy. a, A.O. quartz ocular; b, B. & L. quartz projection ocular; c, e, f, i, n, A.O. two-mirror reflection objectives; g and k, B. & L. two-mirror reflection-refraction objectives; h and j, M.I.T.—Polaroid reflection-refraction objectives; l, m, o, and p, elements of single-spherical-mirror systems. "B. & L." refers to designs by Grey fabricated by Bausch and Lomb Optical Company, "A.O." refers to designs by Kavanaugh fabricated by American Optical Company.

[3] A. Kohler, Zeits. f. wiss. Mik. **21**, 129, 273 (1904).

[4] B. K. Johnson, Proc. Phys. Soc., London **51**, 1034 (1939).

[5] L. V. Foster and E. M. Thiel, J. Opt. Soc. Am. **38**, 689 (1948); L. V. Foster, Analyt. Chem. **21**, 432 (1949).

[6] E. M. Brumberg, Bull. Acad. Sci. U.S.S.R. **23**, 486 (1941); **24**, 33 (1942); Nature **152**, 357 (1943).

[7] D. S. Grey and P. Lee, J. Opt. Soc. Am. **39**, 719 (1949); D. S. Grey, J. Opt. Soc. Am. **39**, 723 (1949).

[8] A. J. Kavanaugh (personal communication).

[9] W. E. Leeds and M. H. F. Wilkins, Nature **164**, 228 (1949).

[10] C. R. Burch, Nature **152**, 748 (1943); Proc. Phys. Soc., London **59**, 41 (1947).

quartz and fluorite, or quartz, fluorite, and rocksalt; hence their use in the infra-red is limited to the region transmitted by quartz.

In addition to suitability for use throughout wide ranges of the spectrum, an important advantage of reflection objectives is that after being focused with visible light they are generally in sufficiently good focus for use in any portion of the spectral range for which they are designed. An important disadvantage is that a portion of the cone of light from the specimen is obscured in passing through any on-axis mirror system, so that the cross section of the used light beam is an annulus. If the linear cross-section ratio of the obscured to the full beam is greater than about 0.3 to 0.4, serious image deterioration may result (Dunham[11]). Several designs keep the linear obscuring ratio below these limits if the full aperture of the system is illuminated. However, unless the full aperture is completely and evenly filled with light, the image quality is likely to be unsatisfactory.

Refracting objectives often give usable images when illuminated by oblique or central beams of relatively small angle. This is not the case for reflection objectives; indeed, a central beam of angular aperture less than that of the obscuring stop does not even pass through such an objective. Hence it is necessary to adjust the illumination much more critically than for refracting objectives. A symmetrical system employing two identical reflection components, one as the condenser and the other as the objective, is often used in order to attain the optimum conditions of illumination. We have found such arrangements to be quite difficult to align, except in the case of the single-mirror system described under Method I. Optimum adjustments are much easier to make if a refracting condenser is used, but a simple Abbe-type condenser is hardly satisfactory for extended ranges of the spectrum because of its chromatic and other aberrations. Conventional achromatic condensers are excellent for the visible region, and we have employed a quartz, fluorite, rocksalt achromatic condenser, designed by Grey, with equally satisfactory results throughout the range 2300–7000A. Only condensers of the reflection type are available for the infra-red.

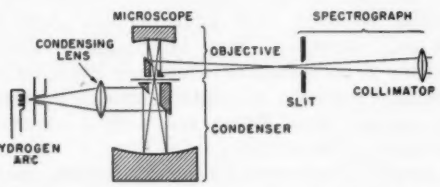

FIG. 2. Diagram of simple system for microabsorption spectroscopy of single crystals, thin solid films, and solutions in microcuvettes, by Method I.

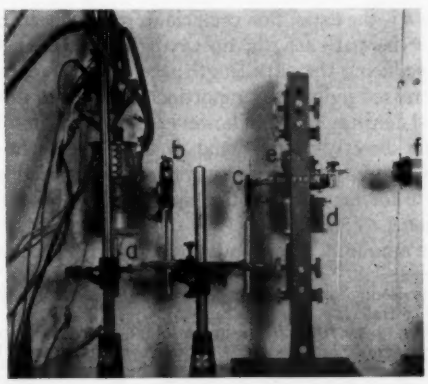

FIG. 3. Photograph of the system shown in Fig. 1. a, hydrogen arc; b, quartz condensing lens; c, diaphragm; d, microscope condenser; e, microscope objective; f, spectrograph. A telescope is used for focusing the microscope image on the spectrograph slit.

[11] T. Dunham, Jr., "The effect of central stops on the resolution of a microscope, I," Special Report No. 2 to the American Cancer Society (July, 1948).

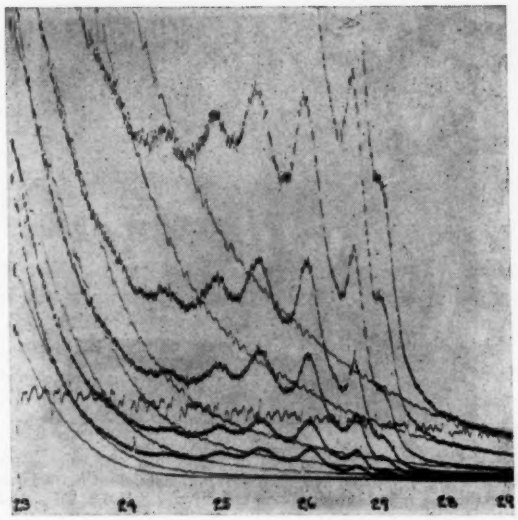

FIG. 4. Densitometer traces of the ultraviolet absorption spectra of a single crystal of phenylalanine together with calibration and wave-length-scale traces. Spectra taken with the system illustrated in Figs. 2 and 3.

Several microscope components of recent design for microspectroscopy are illustrated in Fig. 1. Other components of systems for microspectroscopy are considered in the following sections.

METHOD I. MICROSCOPE FOLLOWED BY SPECTROSCOPE OR SPECTROGRAPH

In the simplest and oldest variation of Method I, a small direct-vision spectroscope is substituted for the eyepiece of a conventional microscope and the spectrum corresponding to the portion of the image that falls on the spectroscope slit is observed visually or photographed. This scheme has long been employed for qualitative visual examination of the absorption spectra

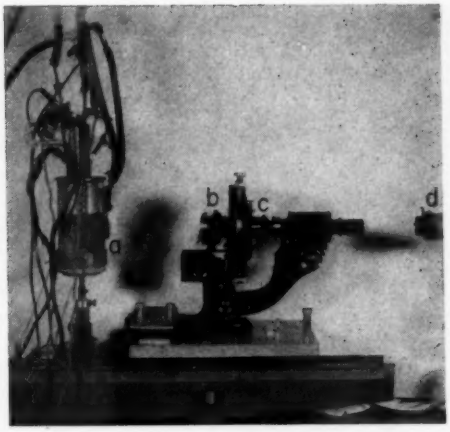

FIG. 5. System similar to that in Fig. 3, but with microscope optics of higher N.A. a, hydrogen arc; b, quartz, fluorite, rocksalt condenser; c, reflection-refraction objective, 2.8 mm E.F.L., 0.72 N.A.; d, spectrograph.

of biological materials. Sjostrand[12] used a Zeiss microspectroscope of this type, with camera attachment, in recent studies of the fluorescence spectra of biological materials. The specimen, excited by ultraviolet by means of a vertical illuminator, was placed on a copper support almost completely immersed in liquid N_2. The Dewar used was of such shape and size that ebullition of dry nitrogen prevented frosting of the objective during the period of exposure (usually several hours).

For such other applications as absorption spectrophotometry or emission spectroscopy, higher linear dispersion is usually required. The image formed by the microscope objective† may then be focused on the slit of a conventional prism or grating spectrometer (for visual observation) or spectrograph (for spectrography). Many variations are possible, of which a few typical examples only will be described.

Figures 2 and 3 show a simple system for ultraviolet absorption spectroscopy of single crystals, solid films, and solutions in microcuvettes. The light source is an improved, hot-cathode, water-cooled hydrogen arc, operating at 10–15 amp., 85 v d.c. (Wyckoff[13]), the discharge being concentrated in an orifice 3 mm in diameter. The microscope (Dunham[14]) employs single spherical mirrors of 25 mm diameter and focal length for the objective and 50 mm for the condenser. The linear obscuring ratio is about 0.25, and the maximum N.A. to satisfy the Rayleigh criterion at 5000A is about 0.16, but this may be exceeded considerably without causing appreciable error in the spectrophotometric results. The image of the specimen is focused on the slit of a small or medium Hilger quartz spectrograph. Absorption spectra are photographed, after which the specimen is moved to one side by the mechanical stage and comparison spectra are recorded on the same plate. Any of the usual methods of photographic photometry[15] may be used, with appropriate modifications; plate calibration by controlled exposure time has been found satisfactory for most work. Figure 4 shows densitometer traces of absorption spectra of a 0.01×0.1-mm portion of a phenylalanine crystal, together with calibration-spectrum traces, obtained with this set-up.

Figure 5 illustrates a similar system employing microscope optics of higher N.A. The condenser shown is the quartz, fluorite, rocksalt achromat previously mentioned; the objective is a Bausch and Lomb reflection-refraction system of 2.8 mm E.F.L., 0.72 N.A. Figure 6 shows absorption spectra of a 0.01-mm² area of a vacuum-evaporated solid film of tyrosine obtained with the above optics and with an American Optical (Kavanaugh[8]) two-mirror reflection objective of 3 mm E.F.L., 0.6 N.A., with corresponding condenser. The absorption curves have not been corrected for the variation in average light path through the specimen as a function of N.A.; they therefore illustrate the higher apparent densities obtained with larger N.A.'s. Appropriate light-path corrections may be computed from the geometry of the system or determined empirically with a standard specimen of sufficient area to be measured both on the micro set-up and with a conventional macrospectrophotometer.

A simple microcuvette for solution spectra, used with this method and Method II, is illustrated in Fig. 7. Since:

$$\log_{10}(I_0/I_x) = kcx, \tag{1}$$

where k is the extinction coefficient, c the concentration, and x the path length, no saving in material ensues from reducing the path length; the concentration must be increased in inverse proportion to maintain the same optical density. There is, however, a decrease in solution volume directly proportional to the decrease in cross-sectional area of the microcuvette as compared with a cuvette for macroabsorption spectroscopy of the same solution.‡

The minimum magnifications, M_w and M_h, necessary,

[12] F. Sjostrand, "Über die eigenfluoreszenz tierischer gewebe mit besonderer Berucksichtigung der sangetierniere," Stockholm (1944).

† An ocular is rarely used since the additional magnification it provides is seldom essential.

[13] H. W. Wyckoff, Quarterly Progress Report, M.I.T. Research Laboratory of Electronics (July, 1949).

[14] T. Dunham, Jr., see J. R. Loofbourow, Growth Symposium 12, 75 (1948).

[15] Harrison, Lord, and Loofbourow, *Practical Spectroscopy* (Prentice-Hall, Inc., New York, 1948).

‡ Thus, a 1×10^{-6}-g/ml solution of adenine has an optical density, $\log_{10}(I_0/I_x)$, of about 1 at 2600A in. 1.0 cm path length. Using a 1-cm macrocell of 5 ml volume, about 5×10^{-6} g of adenine is required for this density. The microcuvette illustrated in Fig. 7 has 1.0 mm path length, and requires a 1×10^{-4}-g/ml solution for the same optical density, but its capacity is only 3×10^{-3} ml, corresponding to 3×10^{-7} g of adenine. Usually, the lower limit of the dimensions is determined by the increased difficulty of manipulation as the size is reduced. Decrease in cross section by an order of magnitude would be feasible in the case illustrated, thus reducing the required amount of adenine to 3×10^{-8} g.

respectively, to fill the width and height of the spectrograph slit are:

$$M_w = w/l_1; \quad M_h = h/l_2, \tag{2}$$

where w is the slit width, h the slit height (used portion) and l_1 and l_2 are the corresponding linear dimensions of the portions of the specimen to be observed. Usually w has a value between 20 and 100μ, but h must be at least 1 mm to obtain a spectrum of usable height unless the plate is moved during exposure or the spectrograph has considerable astigmatism. If, as is usual, $l_2/l_1 < h/w$, M_h determines the minimum magnification to be used. For single crystals, solid films, or microcuvettes, it is seldom necessary to limit l_2 to less than 0.1 mm; hence magnifications of 10 times or less may suffice.§ In the case of biological specimens, l_2 must often be 5μ or less; hence magnifications of 200 or more may be required unless the plate is moved during exposure.

Neglecting diffraction effects, it can be shown that the condition for filling the spectrograph aperture with light is:

$$M+1 \gtrless \frac{(f_1/d_1)}{(f_2/d_2)}, \tag{3}$$

where M is the magnification of the microscope objective, f_1 and f_2 are the respective focal lengths of the spectrograph collimator and microscope objective, and d_1 and d_2 are their respective linear apertures; that is,

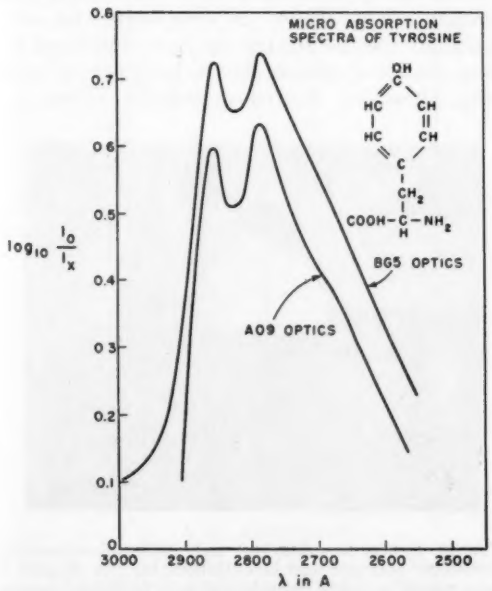

FIG. 6. Absorption spectra of evaporated film of tyrosine obtained with the system shown in Fig. 5 (see text).

§ If the specimen is large enough to cover the spectrograph slit aperture, it may be possible to place it mechanically on the slit rather than to project its optical image thereon. However, it is often more convenient to use the optical method in such instances.

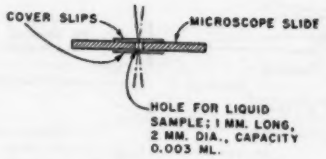

FIG. 7. Microcuvette for liquids and solutions. A second hole (not shown, since it is in a different cross section) is provided to be used for the solvent when solution spectra are determined.

$M+1$ must not exceed the ratio of the aperture number of the spectrograph to that of the microscope objective. The typical "dry" reflection objectives referred to in the description of systems for Method I have N.A.'s of about 0.2, 0.6, and 0.7; the corresponding f/d ratios are approximately 2.5, 0.7, and 0.5. If these objectives are used to illuminate a quartz spectrograph of $f/d=12$, the maximum permissible magnifications for filling the collimator with light are roughly 4, 16, and 23, respectively. In order to approximate the conditions for which the objectives were designed or to obtain sufficiently large images to fill the spectrograph slit, magnifications from two to four times these values must ordinarily be used, with corresponding reduction in the fraction of the collimator that is illuminated. The resulting reduction in resolving power is not ordinarily of consequence in absorption measurements, but the use of presently available microscope objectives with usual prism spectrographs represents a compromise; spectrographs of considerably lower aperture number would be equally satisfactory, and if the aperture number were reduced by increasing the focal length, thus keeping the resolving power constant, the result would be advantageous for some applications.||

Figure 8 illustrates various methods of using simple reflection or refraction microscope objectives for emission spectroscopy. In effect, these schemes are equivalent to the use of a condensing lens of very short focal length, so that a small emission source or a small portion of a larger source may be made to fill the slit and collimator apertures of the spectrograph. Figure 9 shows two iron-arc spectra photographed, respectively, with a quartz condenser of approximately 8.3 cm E.F.L. and a single-mirror microscope of 2.5 cm E.F.L., the magnifications being adjusted to the maximum that would fill the aperture of the spectrograph (Hilger Littrow) in both instances.

METHOD II. MONOCHROMATOR FOLLOWED BY MICROSCOPE

This method has been employed extensively by Caspersson and others for investigations of the ultraviolet microabsorption spectra of biological specimens.[2] A typical set-up as used by Caspersson includes Hg arc and spark sources, a double-prism quartz monochro-

|| There are, of course, some prism instruments and many grating instruments of considerably lower aperture number than f:12, the value given in the example.

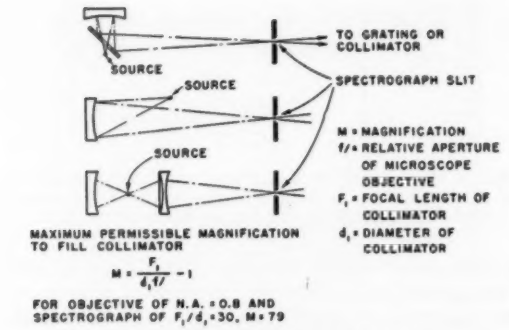
TO GRATING OR COLLIMATOR
SOURCE
SOURCE
SPECTROGRAPH SLIT
SOURCE
M = MAGNIFICATION
f/ = RELATIVE APERTURE OF MICROSCOPE OBJECTIVE
F₁ = FOCAL LENGTH OF COLLIMATOR
d₁ = DIAMETER OF COLLIMATOR
MAXIMUM PERMISSIBLE MAGNIFICATION TO FILL COLLIMATOR
M = F₁/d₁f' − 1
FOR OBJECTIVE OF N.A. = 0.8 AND SPECTROGRAPH OF F₁/d₁ = 30, M = 79

FIG. 8. Simple set-ups for microemission spectroscopy.

FIG. 9. Iron arc emission spectra photographed with macro- and microcondensers. Top: Microcondenser set-up.—Arc magnified 19 times. Area of source used: 166μ^2. Exposure: 6 sec. E.F.L., 2.5 cm. Effective f: No. 1.0. Bottom: Macrocondenser set-up.—Arc magnified 5+ times. Area of source used: 2400μ^2. Exposure: 6 sec. E.F.L., 8.3 cm. Effective f: No. 3.1.

mator, quartz Abbe-type condenser, quartz monochromat objectives (Table I), quartz occulars, photomicrographic camera, photo-cell and electrometer, and rotating sector photometer. Continuous sources are not used because of the narrow spectral band width requirements of the monochromat objectives. Transmission measurements are therefore limited to wave-lengths corresponding to a few Hg arc and spark emission lines. At each wave-length, the transmission of a selected portion of the specimen is measured; the specimen is then moved until a clear portion of the quartz slide and coverslip is imaged on the exit aperture, and I_0/I_x is obtained from the sector setting necessary to reduce the beam to its original intensity.

Cole[16] and others have used a photographic method of photometry for biological specimens in which the entire microscope field is photographed at each wave-length and the plate is calibrated by a rotating sector at the image plane. The position taken up by the sector must, of course, correspond to a clear portion of the field. This method permits transmission determinations to be made for every portion of the specimen included in the photographs, and it obviates the necessity of moving the specimen and returning it to its original position for each new pair of transmission measurements. The method, however, is subject to considerable error as a result of non-uniformity of illumination of the field and other variables encountered in photographic photometry.

The system shown in Fig. 10 incorporates most of the features described above, with some additional ones. As set up in the illustration, it consists of an H_3 mercury arc source (with glass jacket removed), quartz double-prism monochromator, photomicrographic stand equipped with Zeiss quartz optics (or alternatively, with reflection optics) and provided with removable air-spaced calcite polarizer and analyzer prisms, calibrated step-wedge for photographic photometry after the method of Cole, and photo-multiplier tube with output meter and special stabilized power supply for photoelectric photometry after the method of Caspersson. Continuous sources, such as hydrogen arcs, may be substituted when the system is used with objectives that accept comparatively wide spectral band widths. This system has been used primarily for histochemical studies of biological specimens by photoelectric absorption spectrophotometry, employing wave-lengths at the long-wave side of absorption bands for comparison determinations as well as moving the specimen for this purpose as in Caspersson's technique; it has also been employed for transmission measurements of solutions and solid films and for polarized ultraviolet studies of single crystals (e.g. dimethyl thymine). An absorption spectrum of an adenine film obtained with this system is illustrated in Fig. 11.

For special applications in which transmission measurements can be limited to wave-lengths for which satisfactory spectral isolation can be accomplished with filters, the above systems may be simplified by substituting filters for the monochromator (Dempsey,[17]

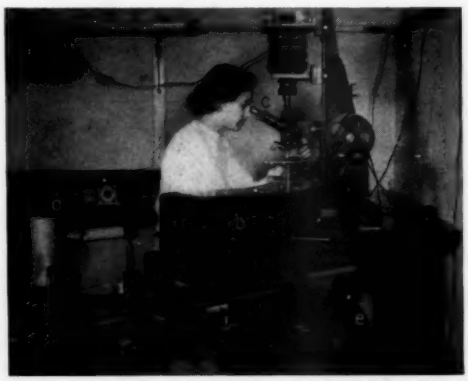

FIG. 10. System for ultraviolet and visible microabsorption spectroscopy, polarized light investigations, etc., by Method II. a, light source; b, quartz monochromator; c, fluorescent eyepiece that can be moved quickly into or out of the light path from the objective; d, photo-multiplier mount; e, regulated supply for photo-multiplier.

[16] P. A. Cole and E. Sutton, Cold Spring Harbor Symposia on Quantitative Biology **9**, 66 (1941).

[17] E. W. Dempsey *et al.*, Anat. Rev. **98**, 417 (1947); see also R. E. Stowell, J. Nat. Cancer Inst. **3**, 111 (1942); M. A. Lessler and H. A. Charipper, Science **110**, 429 (1949).

Pollister,[18] Mellors[19]). In the visible range, this method has been employed considerably for quantitative histochemical studies or microcolorimetry (interference filters, in particular, permit wide choice of wave-lengths); in the ultraviolet, using quartz monochromats with narrow band width requirements, measurements are essentially restricted to 2537A Hg resonance line isolated by $NiSO_4-CoSO_4$ solution filters, but if objectives that will tolerate wider spectral bands (e.g. reflection objectives) are employed, other Hg arc lines may be used.

Recent developments related to Method II include the color-translation system of Land *et al.*,[20] and systems for continuous recording of ultraviolet and visible microabsorption spectra (Sinsheimer[21]).

In the color-translation method, three wave-lengths in the ultraviolet are selected and converted, by intermediate photographic and projection steps, into visible colors. The microscope field appears as a multicolored projection image in which variations in hue correspond to differences in absorption at the selected ultraviolet wave-lengths. This gives, in effect, a three-dimensional presentation in which positions in the image correspond to positions on the specimen and the hue at each point is a function of the ultraviolet absorption, related to the local chemical constitution.

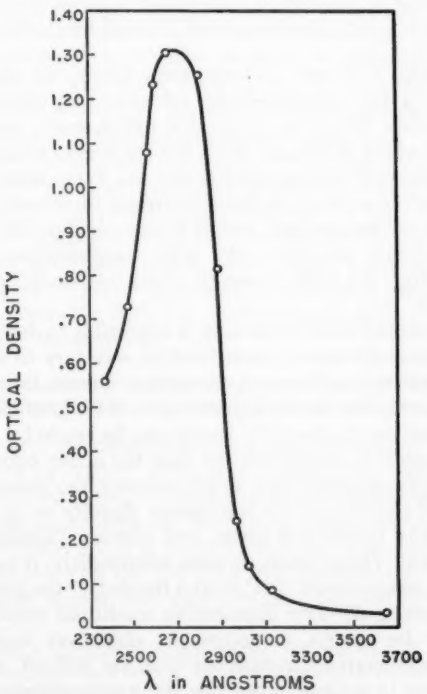

FIG. 11. Absorption spectrum of evaporated adenine film determined with the system illustrated in Fig. 10.

[18] A. W. Pollister and H. Ris, Cold Spring Harbor Symposia on Quantitative Biology **12**, 147 (1947).
[19] R. C. Mellors, Science (to be published); Mellors, Keane, and Streim, Nature (to be published).
[20] Land, Blout, Grey, Flower, Husek, Jones, Matz, and Merrill, Science **109**, 371 (1949).
[21] R. L. Sinsheimer, Ph.D. thesis, M.I.T. (1948).

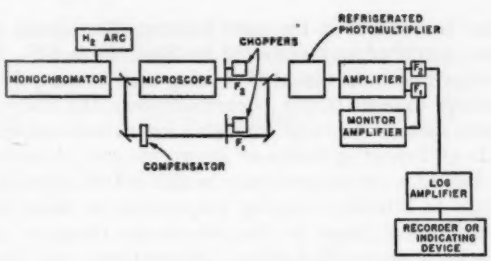

FIG. 12. Block diagram of a system for continuous recording of ultraviolet and visible microabsorption spectra by Method II.

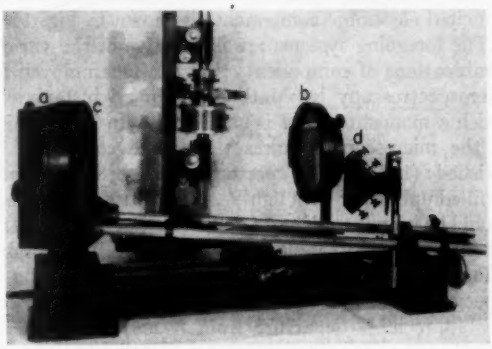

FIG. 13. Wadsworth-mounted grating monochromator, showing reflection microscope in background. a, approximate location of entrance and exit apertures, back of grating mount; b, collimating mirror and mount; c, concave diffraction grating and mount; d, double mirror and slit for isolating spectral region and returning the beam through the system.

A block diagram of a system for continuous recording of microabsorption spectra is shown in Fig. 12 (Sinsheimer[21]). The source is a hydrogen arc (Wyckoff[13]). Spectral isolation is accomplished by a Wadsworth-mounted concave grating monochromator (Fig. 13) in which the light is reflected back through the system so as to result in zero dispersion at the circular exit aperture. The entrance and exit apertures remain in fixed positions at all times, wave-lengths being changed by motion of a double mirror through the spectrum. As in the von Cittert monochromator,[15] the spectral band width may be adjusted readily, and several bands from different regions may be combined if desired. The system is designed for use with an achromatic refracting condenser or reflection condenser and with a reflection objective. Quartz-plate beam splitters are used to separate a comparison beam from the monochromator output and to recombine this beam with the exit beam from the microscope, as shown in Fig. 12. The two beams are chopped at different frequencies (90 and 900 cycles) before they are recombined; after recombination, they are superimposed upon the same aspect of a refrigerated photo-multiplier tube, connected to a cathode follower

that is mounted in the same housing. The signals are then amplified and separated by filters F_1 and F_2, the comparison beam signal (F_1) being used to control the voltage applied to the photomultiplier. The compensated signal is then sent through a logarithmic amplifier into an indicating device or Brown recorder. As shown in Fig. 12, a compensator may be used in the comparison beam to introduce varying attenuation for balancing transmission losses in the microscope elements and specimen mounting medium. Alternatively, the spectrum may be traced twice, once with the specimen image focused on the photo-cell aperture and once with a clear portion of the field focused thereon. A photograph of the principal electronic components is shown in Fig. 14.

The foregoing systems are illustrative of the various combinations of components that can be employed for microspectroscopy by Method II. In all instances in which a monochromator is used, optimum illumination of the microscope requires that three conditions be satisfied: (1) the entire aperture of the objective should be filled uniformly with light, (2) the entire field of view should be illuminated, and (3) the half-intensity spectral band width, $\Delta\lambda$, should be limited to an acceptable value. As discussed previously, the first condition is especially important when reflection objectives are used; any appreciable departure from it may result in intolerable deterioration of image quality. The second condition is particularly important for photo-micrography, photographic photometry by Cole's method, or such applications as color translation. The third condition requires particular restriction of band width when quartz monochromats are used as objectives.

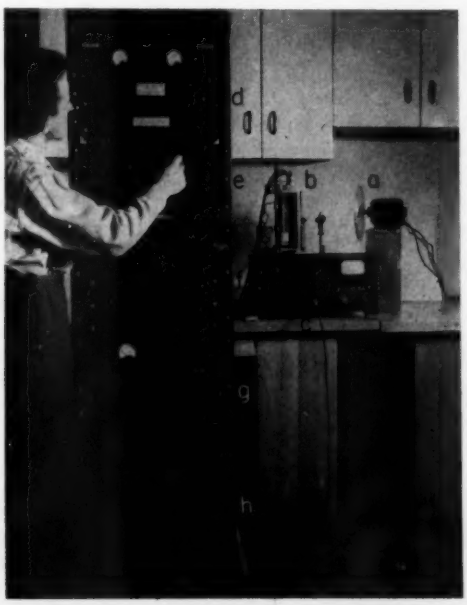

FIG. 14. Electronic components for the system illustrated in Fig. 12.

Since it is often very difficult to meet all these conditions simultaneously, it is important to note that various compromises are acceptable for particular applications. Thus, condition (1) is critical for photoelectric absorption spectrophotometry with reflection objectives, but (2) and (3) are less important, since photometry is generally confined to a small portion of the specimen in the center of the field and acceptable band width is limited only by the spectral resolution desired. With quartz monochromats, condition (3) is critical, but (1) is much less important than with reflection objectives unless the highest possible microscopic resolution is desired; (2) is not important if photometry of the center of the field is of chief interest.

It can be shown that the following equations hold for illumination of a microscope by a light source and monochromator that yield a continuous spectrum of constant intensity as a function of wave-length:

$$(f_1/d_1)/(f_2/d_2)=(d_s/d_f)[1+(1/M)] \tag{4}$$

$$\frac{f_1/d_1}{f_2/d_2}=\frac{\Delta\lambda(dl/d\lambda)}{d_f}\left(1+\frac{1}{M}\right) \tag{5}$$

$$d_f=\Delta\lambda d_1(d\theta/d\lambda)(f_2/d_2)[1+(1/M)], \tag{6}$$

in which f_1/d_1 and f_2/d_2 are, respectively, the aperture ratio of the monochromator telescope and microscope objective. $\Delta\lambda$ is the acceptable half-intensity spectral band width, $dl/d\lambda$ and $d\theta/d\lambda$ are the linear and angular dispersion of the monochromator, d_s, is the monochromator slit width, d_f is the width of the illuminated area of the microscope field, and M is the magnification of the microscope objective. At high magnifications, the quantity $(1+1/M)$ is nearly unity and may be neglected.

From the above equations, it is possible to determine the monochromator characteristics necessary to satisfy any given conditions of microscope illumination; corrections for non-linear dispersion or non-uniform spectral distribution of the light source can be made by usual methods.[22] It may be shown that the above equations are quite general; they apply whether the monochromator illuminates the microscope directly or through auxiliary condensing lenses, and whether "Kohler" or "critical" illumination, or some intermediate, is used.

As demonstrated by Cole and Brackett,[23] the problem of meeting all three illumination conditions simultaneously for quartz monochromat objectives leads to monochromator specifications that are difficult or impossible to achieve in practice if the monochromator is used with a continuous light source. It is for this reason that line sources, such as Hg arcs, Zn or Mg sparks, etc. have been employed exclusively in the use of monochromat objectives.

[22] W. E. Forsythe, editor, *Measurement of Radiant Energy* (McGraw-Hill Book Company, Inc., New York, 1937).
[23] P. A. Cole and F. S. Brackett, J. Opt. Soc. Am. 11, 419 (1940).

The use of a monochromator and light source that satisfy the foregoing criteria is the most important consideration in properly illuminating refraction objectives, but additional problems arise in the illumination of reflection systems because of their critical behavior with respect to uneven illumination. In our experience, unless the area of the source is quite uniform in intensity, and unless this uniformity is maintained at all succeeding aperture planes, adjustments for satisfactory illumination are apt to be exceedingly difficult. Indeed, unless circular apertures are used throughout, the slightest misadjustment may result in sufficient uneveness of illumination to cause image deterioration.

The situation is complicated by the fact that most reflection systems (other than the simple single-mirror system previously illustrated) permit light to pass directly through without striking the mirrors if a critical diameter of illuminated field is exceeded, and by the fact that marked stray light is usually encountered if the aperture is but slightly over filled. Unless the illuminating system is quite carefully designed and adjusted, the performance of reflection systems is generally unsatisfactory. It is much simpler to employ reflection microscope optics in Methods I and III than in Method II. The proper illumination of a monochromator or spectrograph by a reflection objective is comparatively simple, and the direct illumination of the reflection microscope system by a source, or source and condensing lens, involves a minimum of complication.

One other limitation in photoelectric microspectrophotometry arises because of transmission losses in the microscope components, as illustrated in Fig. 15. It should be noted, however, that the measurements shown in this figure were made with typical experimental objectives and do not represent the maximum transmissions obtainable with the best reflection surfaces and dielectric components.

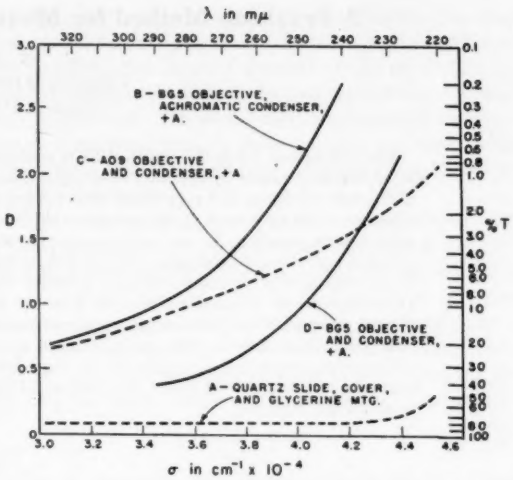
λ in mμ
320 300 290 280 270 260 250 240 230 220
3.0 2.5 2.0 1.5 1.0 0.5 0
D
0.1 0.2 0.3 0.4 0.5 0.6 0.8 1.0 2.0 3.0 4.0 5.0 6.0 10 20 30 40 50 60 80 100
%T
B – BG5 OBJECTIVE, ACHROMATIC CONDENSER, +A.
C – A09 OBJECTIVE AND CONDENSER, +A
D – BG5 OBJECTIVE AND CONDENSER, +A.
A – QUARTZ SLIDE, COVER, AND GLYCERINE MTG.
3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6
σ in cm⁻¹ x 10⁻⁴

FIG. 15. Ultraviolet transmission measurements of various combinations of experimental reflection objectives and condensers. "BG5" refers to the Grey reflection-refraction design listed in Table I, "A09" to the Kavanaugh, dry, two-mirror system listed in the same table, and "achromatic condenser" to the quartz, fluorite, rock salt condenser referred to in the text.

METHOD III. MICROSCOPE FOLLOWED BY MONOCHROMATOR AND RADIATION DETECTOR

Infra-red microspectroscopic investigations have been reported by Barer,[24] Blout *et al.*,[25] and Gore.[26] Method III was used in their studies, the microscope being illuminated by the infra-red source and casting its image on the entrance slit of a conventional recording infra-red spectrometer of the Perkin-Elmer single-beam type. Auxiliary mirrors and apertures may be used for convenience in selecting the portion of the image desired and directing it into the spectrometer slit. Microscope optics designed by Burch[10] were used by the English workers; two-mirror spherical systems of the types listed in Table I have been used in this country.

Single crystals, fibers, biological tissues and cells, and other small samples may be investigated by this method. Specimens upward of 20 to 50μ in diameter are satisfactory in some instances, and the quantity of material involved may be as small as 10^{-7} g. Polarization effects may be investigated by the addition of suitable polarizers.

The problem of satisfactorily matching the microscope objective to the spectrometer is analogous to that discussed under Method I. Since objective design has thus far been conditioned largely by the traditional uses to which microscope objectives are applied in biological photo-micrography rather than by the special needs that arise in various microspectroscopic applications, it is to be expected that the performance of combinations of available objectives and spectrometers should fall short of the ideal. Nevertheless, this newest field of microspectroscopy has already demonstrated the feasibility of obtaining the infra-red spectra of extremely small samples of many substances without undue sacrifice in spectral resolution.

[24] Barer, Cole, and Thompson, Nature 163, 198 (1949).
[25] Blout, Bird, and Grey, J. Opt. Soc. Am. 39, 1052 (1949).
[26] R. C. Gore, Science 110, 710 (1949).

A Precision Method for Measuring Small Phase Differences*

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(Received December 15, 1949)

The well-known diffraction figure of three equidistant slits may be considered as formed by the fringes of equal intensity caused by the outer slits, together with the coherent background from the middle slit, which alternately reinforces and suppresses these fringes. This effect disappears when the focus of the observing telescope is changed so as to cause a geometric path difference of a quarter wave between the middle and outer beams, resulting in two accurate settings of the drawtube inside and outside focus for which the fringes are all of equal brightness. A phase strip in front of the middle slit gives an outward or inward shift of these settings, which measures its path difference with an accuracy of a few thousandths of a wave-length. To compensate for the absorption of the strip the middle slit is made much wider than the outer slits. The effect is reversed if two phase strips are placed in front of the outer slits. By an arrangement with five slits, both possibilities may be alternately switched in, thus doubling the precision. The method is especially valuable for testing larger samples of the material prepared for cutting out the small phase rings to be inserted in microscope objectives. Applications of the same photometric setting to other interference measurements are indicated.

IN manufacturing phase-contrast strips for microscopy, a great need is felt for an exact measurement of the phase difference they produce. Of course it is not feasible to measure the tiny rings built into the microscope objectives directly. It quite suffices to measure a few larger samples, say strips of 2×25 mm, made at the same time and from the same materials as the rings themselves. For the newly invented achromatic and color-contrast phase rings it was especially important to be able to measure a path difference of about 0.25λ with a precision of a few thousandths of a wave-length.

At first sight some reflecting beam-splitter form of interferometer arrangement would seem to be suitable for this. Such is *not* the case, however, for two reasons:

(1) First, the beam splitters with their reflecting surfaces would need to be of uncommon accuracy, and the positioning of the parts would demand an extreme invariability.

(2) The second difficulty is the insufficient accuracy of pointing on an interference fringe, which is ordinarily 1/20 to 1/30 of a fringe.

The first difficulty is avoided if the beam is divided by diffraction. It should be remembered that in the oldest interference experiment, the double slit of Thomas Young, no reflection or refraction is introduced, so that no optical parts are needed. One need not go to this extreme, however. Fizeau used the same principle in 1850, placing the double slit in parallel light between a collimator and a telescope. Rayleigh improved the method in 1910, and instruments of this kind were later manufactured by Zeiss.

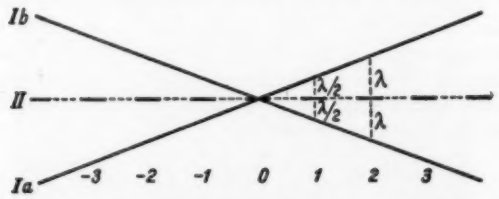

FIG. 1. Fringes in plane *II* caused by the mutually slightly inclined wave fronts *Ia* and *Ib*. Those of odd number are in opposite phase compared to the even ones.

In the matter of the second difficulty, the insufficient accuracy of pointing, the following considerations led to a solution: Rayleigh remarked about fifty years ago that the interference accuracy of 1/10 or perhaps 1/100 of a fringe is far surpassed by photometric settings, such as are of common use in polarimetry. Indeed, the rotation of a sugar solution is easily measured to one minute of arc. This means that the path difference between the right and the left circularly polarized beams is measured to one ten thousandth λ. With special care a precision of 10^{-5} wave-length is attained. Clearly this is made possible by the half-shadow setting criterion, which is a photometric one.

For the lower accuracy of one thousandth λ, therefore, the most primitive photometric setting would do. The general plan of arrangements devised for the purpose is as follows:

One of the beams to be compared by interference is split in two and reunited so as to form ordinary equidistant fringes. The second beam, after having suffered the path difference to be measured, is superposed upon these fringes. The result is that the fringes are alternately reinforced and weakened, provided the second beam is in phase with the first.

Let us go into some further detail with regard to this (Fig. 1). The two halves of beam *I* produce fringes because their wave fronts *Ia* and *Ib* are slightly inclined, the maxima being at the points 0, 1, 2, −1, −2, etc. where the separation of the fronts equals the indicated whole number of wave-lengths. If now the wave front *II* of the second beam bisects the angle between *Ia* and *Ib*, the even numbered fringes 0, 2, etc. will be reinforced, the odd numbered ones weakened by the superposition of II. The reverse will occur if beam *II*

* Presented at the Buffalo meeting of the Optical Society of America, October 1949.

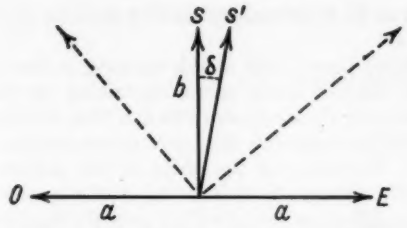

FIG. 2. Vector representation of vibration amplitudes in original fringes, E and O, and interference effect of second beam, S or S'.

has opposite phase. But if beam II has a phase difference of plus or minus $\frac{1}{4}$, its intensity will have to be added everywhere, that is to say that all of the fringes will remain of equal intensity.

This then is the setting criterion: Some compensating arrangement must be changed until the fringes no longer show any alternation of intensity. In order to find the accuracy of this setting, the representation of amplitudes and phases in a vector diagram is more adequate. Let E (Fig. 2) represent the vibration of the beam I in the even maxima—O that in the odd maxima. Superposition of the second beam II with vibration S in quadrature gives equal diagonals right and left. A small retardation δ of S makes them unequal. Their square is found to be

$$a^2+b^2\pm 2ab\delta. \tag{1}$$

Of course the eye is sensitive to the relative difference in intensity of the alternate fringes, which is

$$4ab\delta/(a^2+b^2)=4\delta/(a/b+b/a). \tag{2}$$

This expression has its maximum, a value of 2δ, when $a/b=1$. It is 20 percent less for $a/b=2$ or $\frac{1}{2}$. Clearly the photometric setting is rather unfavorable, as the fringes to be compared are separated by darker parts. Under these circumstances a difference of about 5 percent is to be expected as a discernible minimum. Even this, however, corresponds to $\delta=0.03$ radian or 1/200 of a wave-length.

From this general description the method may seem rather complicated. It is actually, however, very simple when use is made of diffraction in the following way.

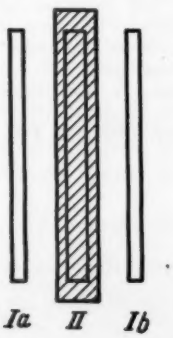

FIG. 3. Three equidistant diffracting slits, of which II may be covered by a phase strip.

A screen with three equidistant vertical slits (Fig. 3) is put on the table of a large spectrometer and illuminated by parallel light. Beam I consists of the light that has passed through the outer slits, beam II of that through the middle one. The phase relations assumed in Fig. 1 are evidently fulfilled. In order to make the amplitude b equal to a, the middle slit can be made twice the width of the outer ones.

The compensating arrangement to be changed until the fringes no longer show any alternation of intensity is simply the drawtube of the telescope. According to a well-known theorem, the optical paths of all three beams will be equal in the true focus, a distance f from the objective lens of the telescope. A simple geometrical consideration shows that at some other distance r the path of the middle beam will be the longer by an amount

$$\Delta l=\tfrac{1}{2}d^2(1/f-1/r). \tag{3}$$

The experiment gives two sharply defined positions of the drawtube, corresponding to $+\frac{1}{4}$ and $-\frac{1}{4}\lambda$, at which positions narrow fringes of equal intensity are seen, the same as if the middle slit were covered up. In the focus, on the contrary, the fringes are twice as far apart, those of odd number having been destroyed by the interference of the beam from the middle slit.

The distance between the two settings evidently corresponds to a half-wave path-difference. In a first set-up this amounted to about 50 mm for green mercury light—the slits being 1.5 mm apart and the focal length of the telescope objective 45 cm. The readings on the millimeter scale of the drawtube needed a small correction to make them proportional to $1/f-1/r$. A small table was prepared for this correction. Alternatively, the scale on the drawtube could easily be made non-uniform so as to give directly the corrected reading.

A glass carrying a sample phase strip may now be placed behind the slits so that beam II is covered by the strip, whereas the beams Ia and Ib pass beside it through the clear glass. Two new settings are then found, and their distance from the zero settings gives a measure of the phase shift caused by the strip. As the phase strips, sampled from materials prepared for use as phase rings in the microscope objectives, are ordinarily absorbing, the resulting weakening of beam II

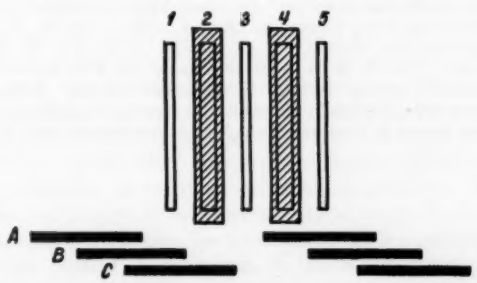

FIG. 4. Five slits, numbers 2 and 4 covered by phase strips, with screen successively in positions A, B, or C.

may be roughly compensated by using a wider middle slit, for instance *four* times the width of the outer ones.

After measuring a number of strips, each in four different wave-lengths, by this arrangement, it was found that the zero reading was somewhat influenced by the glass. Evidently a prismatic error, i.e., a lack of parallelism, has no influence on the difference between *II* and the mean of beams *Ia* and *Ib*. However, the slightest curvature does exert such an influence. It is therefore necessary to determine the zero reading with the glass in place, only shifting it sideways so that the strip no longer covers any of the slits.

The zero setting may be avoided, and the precision at the same time may be doubled, by making a second pointing with *two* strips covering the outer slits. An improved arrangement incorporating this idea is as follows: Five parallel slits 1, 2, 3, 4, 5 (Fig. 4) are cut in a metal screen, slits numbered 2 and 4 being twice as wide as the others. Two strips approximately 2×25 mm are selected for testing from the materials under investigation, and mounted with 2 mm of clear glass between them. For a complete measurement they are placed so as to permanently cover the slits numbered 2 and 4.

A metal screen with a wide opening is then placed behind the first metal screen containing the slits, so as to uncover three adjacent slits at a time. Readings are taken successively with this screen in the positions A, B, and C. The effect of the strips in the position B is evidently opposite to that in the A or C positions, so that the difference $B-(A+C)/2$ gives the double effect.

From 32 complete measurements the mean error was derived for one setting from the discrepancies between the A and C readings, and found to be $\pm 0.0048\lambda$ in complete accord with the prediction. The mean error of a complete measurement, consisting of eight settings is therefore $\pm 0.0017\lambda$. The method is especially useful for strips consisting of relatively thick plastic film (5μ) embedded in a cement of only slightly different index. Because of the averaging character of the measurement, ordinary thin plate glass could be used for mounting the samples.

Adaptation of the new photometric setting criterion to the customary interferometer is being tried.

Symposium on the Theory of Electromagnetic Wave Propagation

A SYMPOSIUM on the Theory of Electromagnetic Wave Propagation will be held June 6, 7, and 8, 1950 at New York University, Washington Square College, New York City under the sponsorship of New York University and the Geophysical Research Directorate of the Air Force Cambridge Research Laboratories.

The program as tentatively scheduled includes the following: **June 6, 1950**—"Field Equivalence Theorems and Their Application to Diffraction Problems," S. A. Schelkunoff (Bell Telephone Laboratories); subject to be announced, R. D. Spence (Michigan State College); "Waves on Inhomogeneous Cylindrical Structures," R. B. Adler (Massachusetts Institute of Technology); "Criteria for Discrete Spectra," K. O. Friedrichs (New York University); "The Diffraction Theory of Gaussian Optics," H. Bremmer (Philips' Research Laboratories, Holland); "Wiener-Hopf Techniques and Mixed Boundary Value Problems," S. N. Karp (New York University). **June 7, 1950**—"The W. K. B. Approximation Method," R. E. Langer (University of Wisconsin); "The W. K. B. Approximation as the First Term of a Geometrico-Optical Series," H. Bremmer (Philips' Research Laboratories, Holland); "Remarks Concerning Propagation of Plane Waves in Stratified Media," S. A. Schelkunoff (Bell Telephone Laboratories); subject to be announced, N. C. Gerson (Air Force Cambridge Research Laboratories); "Oscillations of the Atmosphere," B. Haurwitz (New York University); "The Theory of Scattering of Radio Waves in the Troposphere and Ionosphere," H. G. Booker (Cornell University); "Mode-Coupling in the Ionosphere (*Z*-Trace)," J. W. Cox (Defense Research Board, Canada). **June 8, 1950**—"Propagation in Spherical Multilayers," N. Marcuvitz (Brooklyn Polytechnic Institute); subject to be announced, O. E. H. Ryebeck (Chalmers University of Technology, Sweden); "Relationships between Alternative Formulations of the Anomalous Propagation Problem," B. Friedman (New York University); Subject to be announced, H. Levine (Harvard University); "On Systems of Linear Equations in the Theory of Guided Waves," W. Magnus (California Institute of Technology); "Wave Propagation over Rough Surfaces," S. O. Rice (Bell Telephone Laboratories); "Evaluation of Integrals Associated with Wave Motion in Dispersive Media and the Formation of Transients," M. Cerrillo (Massachusetts Institute of Technology).

There will be no registration fee for attendance at the Symposium. Further information may be obtained from Morris Kline, 45 Astor Place, New York 3, New York, Room 514.

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Color Phase-Contrast Microscopy: Requirements and Applications*

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For the use of color phase contrast in microscopy, which was first discussed in the Spring of 1948, several difficult requirements must be met for the attainment of maximum brilliance and usefulness. The system itself is one in which a controlled chromatism of the phase plate is sought instead of the customary control of phase difference at one wave-length only. The thickness and dispersion of the phase ring in relation to the general aperture can be such that zero phase contrast will occur at one or more wave-lengths of transition in color sensation. If the phase contrast is made high but opposite in several spectral zones of relatively pure color sensation, striking and beautiful microscopical images are formed with objects of low inherent contrast. The greatest advantage of the system lies not in the beauty of the images, but in the clear distinction which is afforded between effects caused by scattering or general absorption and those caused by small differences of refractive index or thickness.

INTRODUCTION

AN object, when viewed by phase-contrast microscopy,†[1] is illuminated by radiation of comparatively restricted angular extent. This is accomplished by using an ordinary microscope condenser supplemented by a pattern diaphragm located approximately at the lower focal plane of the condenser. From each point in the opening of the pattern diaphragm, approximately plane waves pass through all parts of the object where they are either (1) transmitted still as approximately plane waves, in which case they form an image of the diaphragm approximately in the back focal plane of the objective, or (2) diffracted in all directions, in which case they illuminate, in greater or lesser degree, portions of the objective aperture outside the image of the diaphragm. If an optical sheet of varying refractive index at the upper focal plane of the objective conforms in pattern and position to the image of the pattern diaphragm and if those areas of the sheet lying within the image formed by the directly transmitted light have optical paths uniformly greater or less than other areas of the sheet, the basic requirements of phase contrast are met. When the optical paths in the different areas of the sheet, "phase plate" or "phase element," differ by such amount that the directly transmitted light is *advanced* relatively to the diffracted light by one-quarter wave-length or by one-quarter wave-length plus or minus a whole number of wave-lengths, then local portions in an object which have slightly higher refractive indices than their surround appear darker than the immediately adjacent areas, while local portions of slightly lower refractive indices appear brighter. Conversely, if the directly transmitted light is *retarded* relatively to the diffracted light by one-quarter wave-length plus or minus a whole number of wave-lengths, portions in the object of slightly higher refractive index will be brighter and those of lower index will be darker than the immediately surrounding areas.

In practice the pattern diaphragm is almost always in the form of a narrow annulus of transmission. Almost any other shape could be employed and, indeed, for certain specialized uses, other shapes may be preferred. The narrow annulus in the condenser requires that in the objective there be a ring of one substance of a definite thickness conjugate with it and that other parts of the objective aperture have a constant thickness of another substance, the difference between the optical properties of the two substances causing the phase shift. Often the two substances are air and evaporated magnesium fluoride.

Little attention has been spent upon the nature or width of the "immediately surrounding" portions of the image mentioned above. In the original development of phase-contrast microscopy and its theory, phase gratings served as typical objects. There the area of depression immediately surrounding any elevation in the grating was flanked by another elevation. Effects a short distance from any discontinuity tended to be obscured by the effects of more adjacent discontinuities. The phenomena with other types of object were derived by extension from those with phase gratings. The extent of changes in brightness near the image of optical discontinuities in the object has considerable importance to the present investigation. A photo-micrograph by phase contrast of several crystals of sodium chloride, closely adjacent to one another, is shown in Fig. 1.

* Presented at the Buffalo meeting of the Optical Society of America, October 1949.

† Zernike, the inventor of phase contrast, was also the proponent and first experimenter with color phase contrast.

[1] F. Zernike, Zeits. f. tech. Phys. 16, 454 (1935); Physik. Zeits. 36, 848 (1935); Physica 9, 686; 974 (1942).

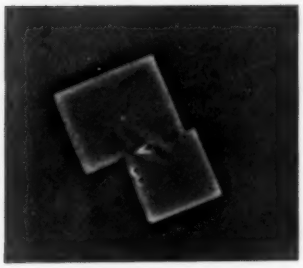


FIG. 1. Crystals of sodium chloride, $N_D=1.5443$, in a liquid, $N_D=1.5476$. Magnification 200×, objective N.A. 0.40, sodium light.

FIG. 2. Pyrex glass film, 1.2μ thick $N_D=1.4752$ in right-half of field immersed in liquid $N_D=1.4761$. Magnification 200×, objective N.A. 0.40, sodium light.

Their refractive index is slightly lower than the oily liquid in which they are immersed and, since the phase-contrast objective was of the first type mentioned (light path in image of pattern diaphragm $\frac{1}{4}\lambda$ shorter than in rest of aperture), they appear brighter than the immediately surrounding part of the image. It will be noted that the center of the cubical crystals is very little, if any, brighter than those portions of the image which are comparatively remote. It is as though some of the light passing the edge of the crystals had been transferred across the boundary and had appeared in the image space within the image of the crystals. The fact that the intensity of illumination remote from the image of a boundary is the same on both sides is even more clear in Fig. 2. A film of Pyrex glass about one-half micron thick covers the entire right-half of the field. It is immersed in, and surrounded by, a mixture of mineral oil and 1-bromonaphthalene which has a refractive index about 0.020 higher. Equivalent areas at the centers of the right and left halves of the field can be easily compared.

INFLUENCE OF DISPERSION CHARACTERISTICS ON NEUTRAL PHASE CONTRAST

The evaporated films of magnesium fluoride, which are widely used in conjunction with air to cause the phase shift in phase-contrast objectives, have refractive indices which decrease with wave-length about as indicated by the continuous curve in Fig. 3. If the phase-contrast effect for such films were to be equal for all wave-lengths, it would be necessary for the index of refraction minus one, divided by wave-length, to be equal at all wave-lengths. This cannot be true for any known substance used in conjunction with air. To achieve such a result, the refractive index of the substance would need to be as represented by the dotted line in Fig. 3 where the slope is opposite to that of everything known.

When two different substances are used to form the ring and the matrix areas, it is easy to satisfy to any desired degree, the condition

$$(N_r-1)t_r-(N_m-1)t_m=\pm\tfrac{1}{4}\lambda\pm a\lambda$$

where t_r and t_m are the thicknesses of the ring and matrix substances which have refractive indices N_r and N_m at wave-length λ and a is a whole number. It is nearly as easily attained when the thicknesses are equal and the condition becomes

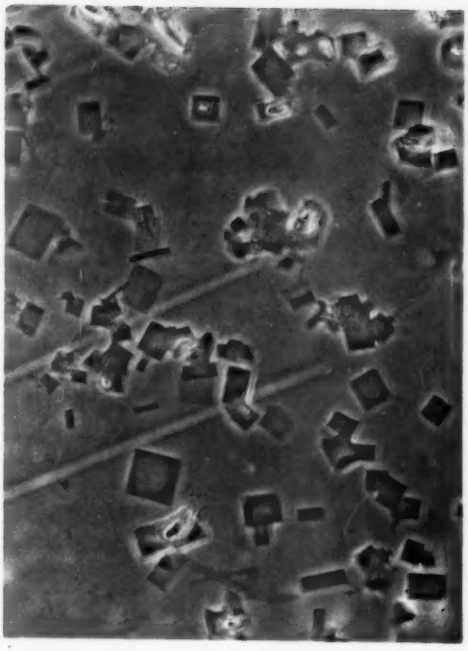
$$(N_r-N_m)t=\pm\tfrac{1}{4}\lambda\pm a\lambda.$$

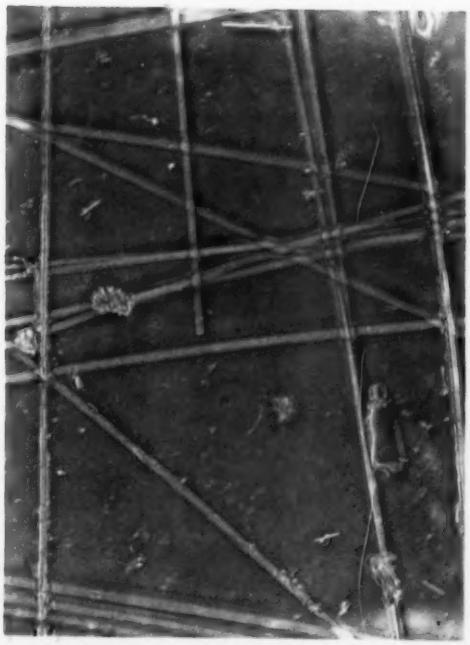
The refractive indices of two plastic substances represented by the thin full curves in Fig. 4 are almost exactly right. The substance with a little higher index of refraction has a little lower dispersion and the vertical distance between the curves is proportional to the wave-length. Phase-contrast equipment made according to this plan would be equally efficient with all colors. The extent to which standard equipment loses effectiveness because of chromatic faults is not certain, but it may be serious.

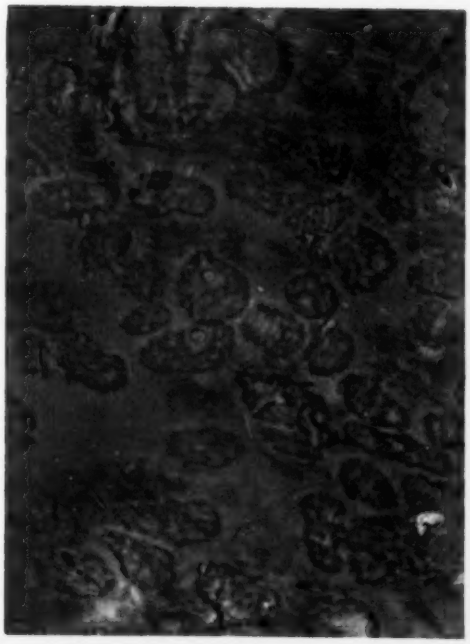
PRODUCTION OF COLOR CONTRASTS BY DISPERSION OF PHASE PLATES

If one could find substances which were like those represented by the thin full lines of Fig. 4, except that the long and short wave-length ends were exchanged—as in curves A and B of the figure—phase-contrast effects would be opposite for colors on opposite sides of the cross-over. The cross-over is illustrated at 0.55μ. If A represented the substance of the ring and B that of the matrix in a phase element, a particle, a small area in an object, which was slightly higher in index of refraction than its environment would appear darker if red light alone were used and would be surrounded by a band brighter than areas remote from the particle. On the other hand, with blue light the particle would be bright, surrounded by a dark halo. With white light it would appear blue and be surrounded by a red zone. The background would be gray. If the cross-over were shifted from a wave-length of 0.5μ to 0.6μ, the area would change from a deep blue with yellow halo and gray background to bright cyan with deep red halo and gray background. It is obvious that, if the refractive index of the particle were made slightly lower than its environment, or if B instead of A were used for the ring, these effects would all be reversed.

This ideal manifestation of color phase contrast is, of course, impossible. Dispersion curves do not exhibit







the immaculately abrupt changes which are represented in curves *A* and *B*. Usefully close approach to such effectiveness is theoretically possible, however, by several independent techniques as well as by combinations of two or more of them. To attain effectiveness of that order, however, would require detailed investigation of refractive indices and dispersions, still unclassified, and perhaps undetermined, of many different substances. Not only is it necessary that the optical properties of at least two substances be correctly balanced (they do not need to be pure compounds)—they must also be chemically and mechanically stable in contact and capable of fabrication. There are at least four of these independent techniques.

1. While curve *B* of Fig. 4 is unlike any dispersion curve with which we are familiar, forms similar to *A*, but milder in their inflections, are fairly common. By greatly thickening the phase shifting element, a substance with simple Hartmann-type dispersion could be used in conjunction with another material of which the dispersion curve was nearly parallel except for a slight displacement well within the visible spectrum. It should be possible to utilize thicknesses as great as several millimeters in such a device.

2. Two substances having ordinary dispersion curves that cross at or near the optic centroid should give reasonably satisfactory results. When the phase elements have such thickness as to produce maximum effects more or less central to the two halves of the spectrum, higher efficiency is attained than one tends to anticipate. The efficiency of the phase-contrast microscope has the general form of the sine of the relative retardation, $\sin[(N_r-N_m)t/\lambda]$, where each successive whole-number value of the term $(N_r-N_m)t/\lambda$ constitutes a period. The sine, of course, departs rapidly from zero and maintains a high proportion of its maximum value during much of each half-period.

3. If the refracting elements of the phase plate have the same difference in index at all wave-lengths, the difference in path expressed in waves is inversely proportional to the wave-length. By causing the diffracted light to be advanced or retarded a whole number of half-periods at one part of the spectrum, the sine, reaching maximum positive and negative values at successive odd quarter-periods, can attain these opposite values at other places well within the visible spectrum. Thus in Fig. 5, curve *A* is a plot of $\sin[(N_r-N_m)t/\lambda]$ where N_r-N_m (the index of refraction of the ring minus that of the matrix) is independent of wave-length and where $(N_r-N_m)t/\lambda$ is 2 at wave-length 0.54μ. Using a phase element according to this specification, the high index side of an object boundary would appear bright at all wave-lengths where the curve was positive. Portions in objects that were higher in index of refraction would appear blue relative to the field and portions of low index would appear orange. Curve *B* of Fig. 5 is plotted for the condition where $(N_r-N_m)t/\lambda$ is 1 at 0.54μ. Objects would appear much the same except that low index portions would appear redder and high index portions would be more violet. An interesting variant is that in which the portion of the spectrum selected for zero contrast is made to correspond with a half-period of retardation. Thus if $(N_r-N_m)t/\lambda$ is 1.5 instead of 1.0 or 2.0, the sine is still zero, the phase-contrast effect at the selected part of the spectrum remains null, but as with curve *C* of Fig. 5 there are pronounced effects in the red and blue parts of the spectrum which are opposite in sign to curves *A* and *B*. Of the plans for achieving color phase contrast by phase shifts alone, this seems the most promising. The color stimulating areas under the right and left lobes of this curve are noticeably greater than when $(N_r-N_m)t/\lambda$ is 1 or 2 at the middle of the spectrum and much greater than those cases where it is less than 1 or greater than 2.

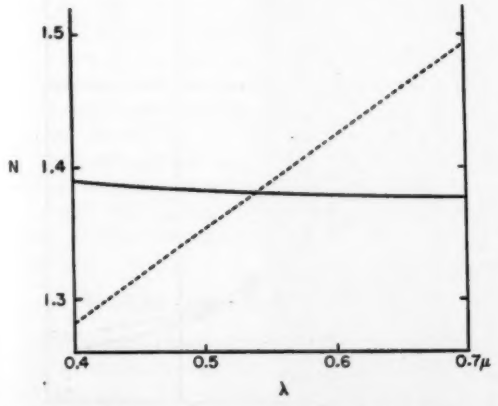


FIG. 3. The full curve represents the refractive index of evaporated magnesium fluoride according to the data at hand. The dotted straight line shows the dispersion that would be necessary for a single evaporated film to have equal efficiency at all wave-lengths (achromatic phase contrast).

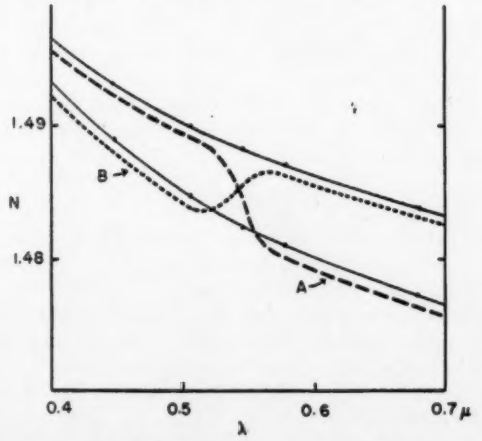


FIG. 4. The thin full lines represent refractive indices of two actual plastic substances for which $N_1-N_2 \propto \lambda$. Curves *A* and *B* represent hypothetical substances from which ideal color phase-contrast plates might be made.

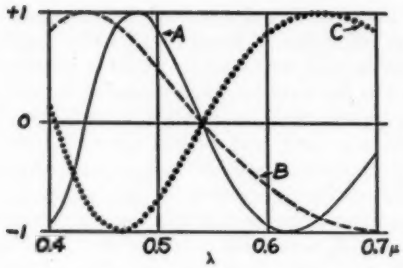


FIG. 5. Values of $\sin[(N_r-N_m)t/\lambda]$ when $(N_r-N_m)t/\lambda$ is 2 (curve A), 1 (curve B), and 1.5 (curve C) at 0.54μ, N_r-N_m being independent of wave-length.

Obviously, if $(N_r-N_m)t/\lambda$ is negative instead of positive, all effects will be the same except that there will be interchange of colors corresponding to opposite sides of a high-low index boundary in the object.

4. By making the ring and matrix substances of different, though uniform, thicknesses and immersing them in a third substance, the number of potentially controllable variables is greatly increased. Three different dispersion curves can be balanced against each other, the effective thickness of each substance having an independent value. Problems of selection of mutually stable materials (with the right optical properties) and of fabrication of parts are obviously much greater under this system.

It should be possible to combine the systems mentioned in the foregoing paragraphs in conjunction, although we have not as yet experimentally explored these opportunities. It is noteworthy, for example, that by both methods 2 and 3 the oscillations of the phase function tend to be more congested at the blue end of the spectrum. This effect is more pronounced for method 2 than for method 3. If, therefore, the principal phase shift were accomplished by method 3, but partly compensated by method 2, such relative congestion could be nearly or entirely eliminated. Similarly the particular forms of unusual dispersion curves which are the basis of method 1 can be considered in connection with method 2 or 3. Implicitly, method 4 can be used with any combination of the other methods.

EXPERIMENTS

In 1948 the third author showed the results of some of his first experiments in color phase contrast.[2] The phase element which he employed at this time had a ring of cellulose nitrate with high dispersion plasticizer. This was mounted in balsam between glass disks. Those who looked at real objects through the microscope were impressed by the possibilities and potential brilliance of the method.

[2] F. Zernike, Report of the National Academy of Sciences, 31(A) (1947–48). Transparencies of miscellaneous biological objects, made by color phase contrast, were shown but, due to the necessities of the occasion, explanation was very brief.

The present work has involved a wide variety of materials and methods of assembly, most of which, it must be confessed, attained little success. Rings of cellulose triacetate mounted in a plasticized matrix of polyvinyl butyral seemed very promising. The plasticizers were used to adjust both the refractive index and the dispersion of the matrix. Unfortunately, a small amount of the plasticizers, listed as incompatible with cellulose acetate, tended moderately quickly to diffuse from the polyvinyl butyral into the acetate. This probably represented a small change in composition. With the thick phase elements necessary for this combination, however, extremely slight diffusion of that kind would quickly spoil the device.

Rings and disks of optical glass, of which the refractive indices were most accurately known, seemed the surest permanent means of attaining the desired results. The long and tedious nature of shop work in this field greatly limited the power to proceed on the basis of observation to other modified devices. Accordingly, this approach was interrupted when several other methods began to produce results of unquestioned usefulness.

Gelatin in Plastic Matrix of High Dispersion

Gelatin was dissolved in warm water, colored with a neutral dye, and carefully filtered. A measured amount of the solution was pipetted on to a level chromium plated surface where it was spread out to cover one square decimeter. After the film had dried and peeled

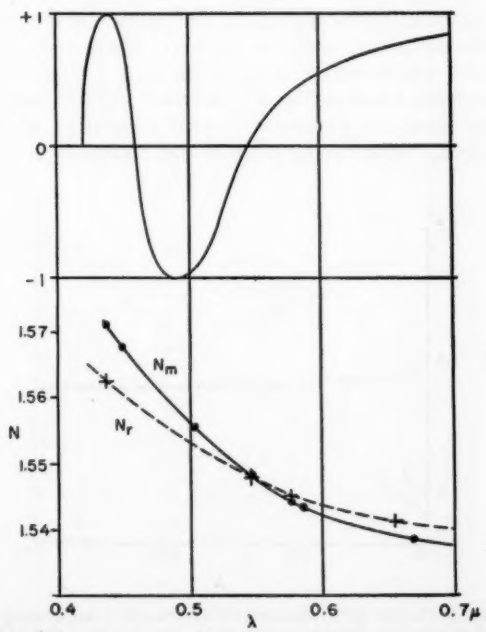


FIG. 6. Dispersion curves of dyed gelatin film (dashed line) and complex plastic (full line) crossing at 0.555μ. Upper curve represents $\sin[(N_r-N_m)t/\lambda]$ using a gelatin ring 45μ thick.

off, it was dried for several hours at 80°C and its refractive indices were measured. For this purpose the film was pressed with a black rubber cushion against the hemisphere of an Abbe-Pulfrich crystal refractometer. Methylene iodide was the contact liquid on both sides of the gelatin film. The dispersion curve is shown as a dotted line in Fig. 6.

The matrix was made with 16 percent polyvinyl acetate, the rest being a complex plasticizer of high dispersion. The plasticizer was prepared in two parts having high and low refractive index. For simplicity the plastic was introduced into both parts. The low index portion thus was simply plastic with 84 percent octyl phthalate. For the high index portion 2-nitrodiphenyl, 1,3-dinitrobenzene, and 2,4-dinitrochlorobenzene were used in the ratio 5:4:1. This mixture would remain fluid for days after melting. With it there was compounded 16 percent of the plastic. On the basis of their refractive indices after they had been kept at 80°C for several hours, these two portions were melted together in several experimental ratios and finally compounded so that the refractive index at 0.555μ matched that of the gelatin. The dispersion curve is portrayed as a solid line in Fig. 6. With a gelatin ring 45μ thick and this matrix, the function $\sin[(N_r-N_m)t/\lambda]$ has the properties of the upper curve in Fig. 6.

Some of the gelatin film was cemented to a microscope slide with wax, the slide clamped to the revolving stage of a microscope, and the corner of a broken safety-razor blade held with a micromanipulator. By observing under low power with a micrometer eyepiece, the position of the razor blade could be controlled and neat rings readily cut by revolving the stage of the microscope. These rings were made 3.1 mm in outside and 2.6 mm in inside diameter. At the same time, concentric larger rings were cut to be used at the rims of the glass plates in order to assure parallelism.

The two rings, the smaller working ring and the larger spacing ring were cemented in place on one flat glass disk of such diameter that it could be inserted in objectives. The matrix mixture, thinned with a little solvent, was used as the cement. A drop of melted matrix compound was applied so that it would flow over the phase ring, the second flat glass disk laid on top, the assembly put between the cushioned jaws of a pinch clamp, and everything was placed in an oven at 80°C until full optical contact had been secured and excess matrix compound squeezed out through the edges.

Multiple Order Retardation Plates

Experimental phase elements in which unplasticized cellulose triacetate was used for the ring and unplasticized polyvinyl butyral for the matrix have been attempted with one and two periods of phase change. These have not been very satisfactory, probably because the small difference in index of refraction (−0.0144) and the necessary thicknesses (39μ and 77μ) caused small changes in refractive index, especially local changes, to be extremely deleterious. The possibility of using phase shifts of 1.5 periods, like curve *C* of Fig. 5, did not become apparent until later. Very little work has been done in this way. One phase element has been made, however, using a gelatine ring and a matrix of polyvinyl butyral on which refractive index and dispersion had been adjusted with butyl phosphate and 2-nitrodiphenyl.

Description of Color Plate

All observations have been made with essentially white light and objects that were, with one exception, either uncolored or neutrally absorbing. Photo-micrographs of such test objects are shown in the color plate. These pictures were all made with an objective of 20 diameters magnification and numerical aperture 0.40. Final magnifications are 200 diameters except for the lower right photo-micrograph which has a magnification of 400 diameters.

The photo-micrograph in the upper left corner shows crystals of the isometric salt, sodium chlorate (refractive index about 1.515) and fiber glass (refractive index about 1.528) mounted in a liquid with an intermediate refractive index. This was chosen as a test object because it was possible accurately to know the physical properties—refractive index, shape, etc.—of all parts. The curve of phase effects is as shown in Fig. 6, as a result of which the fiber glass is brighter by red light and darker by blue light. Also presumably it is brighter by violet light so that the red areas have a slightly magenta tint. It will be noticed that the spaces in the immediate vicinity of the fibers are blue. The crystals, on the other hand, since they have an index of refraction lower than the liquid are darker by red light and brighter by blue. They appear blue and the space near them is pink.

The upper right photo-micrograph shows an ultra-thin section,[3] mounted in balsam, of the protozoan, Spirostomum ambiguum. The animal has been very faintly stained with fuchsin which gives certain parts a slight magenta color. The pellicle, outer wall, and the polynucleus have refractive indices slightly higher than the balsam and appear red, principally because of the slight increase of refractive index and only secondarily from the staining. Other discontinuities, stained the same color as the pellicle, appear greenish blue when their refractive indices are lower than that of the balsam. Refractive index differences are manifested as differences in color but are superimposed upon the feeble colors that have resulted from staining.

The lower right photo-micrograph approaches the limit of thinness for which this or other optical methods are serviceable. It is an extremely thin section of an onion root tip. It will again be noted that, where a

[3] Made by Emil Borysko according to the method of Newman, Borysko, and Swerdlow, J. Research Nat. Bur. of Stand. 43, 183 (1949); RP 2020.

structure appears blue, the space in the immediate vicinity is pink, and where a structure is red, the space around it is blue.

That the apparatus with which the pictures were made is still imperfect is shown by the photo-micrograph of glass fibers in the lower left corner. As their refractive index is higher than the immersion medium, they should appear pink with greenish blue edging. The room temperature when the picture was made was about 10°C above normal, however. As a result, a differential change in refractive index between the ring and the matrix of the phase element had occurred, shifting the crossover of Fig. 6 to shorter wave-lengths. The fibers are therefore more yellow than pink and the edging is blue.

COMMENTARY

The objects shown in the accompanying photomicrographs would all be nearly invisible by most forms of microscopical examination. In the protozoan pictures, the texture in the interior of the cells cannot be interpreted by ordinary central illumination. With neutral phase contrast the interlocking dark and light areas are very confusing. The color phase procedure, however, clearly delineates everything that is subject to resolution. Ultimate interpretation of structure is naturally still subject to the judgment of the observer.

It is not clear what is the best part of the spectrum to have zero contrast. In the apparatus that has been described, an attempt was made in all cases to locate it near the optic centroid. As a result, except in one experiment, regions of differing refractive index have nearly the same brightness and adjacent areas in the image differ only in chromaticity. This was done with the purpose causing inherent absorptions of a sample to be manifested as differences in brightness while differences in structure or refractive index would be shown by other aspects of color. In the exceptional experiment, that with the glass wool fibers (lower left), there is some indication that the red to greenish blue contrast is more valuable than the yellow to blue.

The capacity of this type of phase-contrast equipment to discover threshold differences in refractive index has so far been less than that achieved with neutral phase contrast. Whether this is inherent in the system—a discrimination where all light is decreased or increased being more sensitive than a relative change in brightness in different parts of the spectrum—is not clear. Certainly the curves of Figs. 5 and 6 do not indicate maximum efficiency. The sines do not change abruptly between plus one and minus one. Light sources composed of three mixed nearly monochromatic primaries located at the peaks or minima of curves might be used to augment the sensitivity of the color phase-contrast method. While this is a possible development, the simultaneous utilization of several of the methods mentioned seems more practical in the long run.

A curiously complementary character of this color phase-contrast method to the dispersion staining with phase contrast described by Crossman[4] should be mentioned. In his work, neutral phase contrast is used with objects in which index is deliberately nearly matched while great differences in dispersion are introduced. It is obvious that his method calls for specialized balance in the object. In the present work such balance is transferred to the apparatus.

For reasons of convenience, most of the experimental work with color phase contrast has been performed with phase elements having neutral absorption of light in the phase ring. This causes a reduction in intensity of the direct relatively to the diffracted light which contributes to any part of the image. Consequently, as Zernike[1] emphasized in connection with neutral phase contrast, those structures which are visible by reason of absorption or extreme scattering tend to be suppressed in the image, while those structures which have slight refractive differences from their surround are enhanced. Color phase-contrast effects can be achieved without suppression of amplitude effects if a very narrow phase ring without absorption is used. This reduces the intensity of the direct but not the diffracted light and causes the amounts which contribute to any part of the image to be more nearly equal. Because the halo surrounding structures tends to be much broader in such cases, the effect of the direct light compared with the diffracted light is much weakened at any point in the image and the vectorial optimum of nearly equal amplitude in interfering beams is attained.

The color effects of color phase contrast can thus be superimposed upon differences in darkness naturally exhibited by a sample. They are deliberate optical artifacts which, by being directly correlated to optical density, give an intimation of chemical constitution. It seems probable that the greatest potential advantage of the system lies not in the beauty of the images, but in the clear distinction between effects caused by scattering or general absorption and those caused by small differences of refractive index or thickness. But it must not be forgotten, that only after biologists, clinicians, mineralogists, and other users of the light microscope have had a chance to put it into actual service, will its ultimate importance be known.

The aid, through consultation and laboratory assistance, of Samuel G. Weissberg of the Organic Plastics Section, Barbara M. Sullivan and Max Swerdlow of the Crystals and Microstructure Section, Earl F. Webb of the Optical Instruments Section, H. I. Feinstein of the Organic Chemistry Section all of the National Bureau of Standards, and Harold E. Finley of Howard University have been especially valuable.

[4] G. C. Crossman, Science **110**, 237 (1949).

Letters to the Editor

Two Infra-Red Sensitive SrS Phosphors with Zn Dominant Activator

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December 14, 1949

THE infra-red sensitive phosphors have been the subject of extended research in the recent years.[1–6] According to experiments carried out by the author, it was found that Zn can be used as a dominant activator in the following interesting combinations SrSZnBi and SrSZnSm.

(a). SrSZnBi: *Composition:* SrS 1g, Zn 800γ, Bi 80γ, Li_2CO_3 0.02 g. The emission of this mixed phosphor appears on Fig. 1,

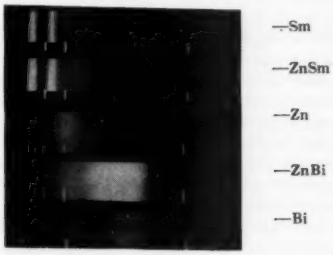

FIG. 1. Emission bands of various activators in SrS phosphors. Emission bands of mercury: (a) 5790A; (b) 5460A; (c) 4358A.

where the emission of single SrSZn and SrSBi are also shown. This mixed phosphor, under the influence of infra-red radiation shows a stimulation of the emission band of the Zn while simultaneously the Bi emission is quenched. Figure 2 shows how the decay of emission bands of Zn and Bi changes if an infra-red radiation acts for a very short time period. The decay curves are obtained by means of a rotating cylindrical phosphoroscope with three windows on its cylindrical surface. One of the windows is for the ultraviolet exciting light, the second for the infra-red

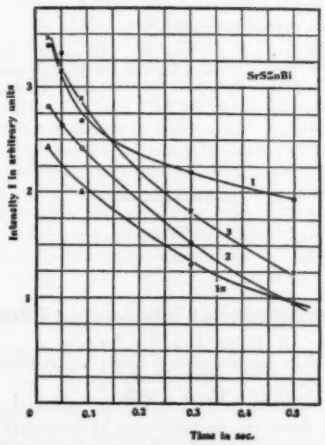

FIG. 2. Phosphorescence decay of SrSZnBi phosphor. Separate Zn and Bi emission bands. 1. Decay of Bi emission band. 1α. Decay of Bi emission band after a momentary action of infra-red rays; 2. Decay of Zn emission band. 2α. Decay of Zn emission band under conditions same as in 1α.

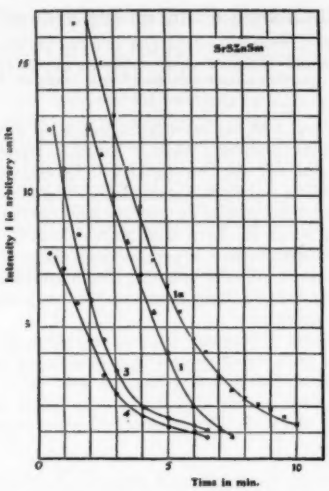

FIG. 3. Phosphorescence decay of SrSZnSm phosphor. Separate Zn and Sm emission bands. 1. Decay of Zn emission band. 1α. Zn emission decay after 5-sec. infra-red rays action. 2. Decay of Sm emission band. 2α. Sm emission decay under conditions same as in 1α.

radiation and the third for the photo-cell. The time lag of the Zn stimulation of the emission band is relatively small. The long duration and brightness of the total phosphorescence of this mixed phosphor are important, as compared to SrSBi and SrSZn phosphors.

(b). SrSZnSm: *Composition:* SrS 1g, Zn 800γ, Sm 200γ,* Li_2CO_3 0.02 g. Figure 1 shows the emissions of SrSZnSm and of SrSZn and SrSSm. This mixed phosphor is distinguished by a high time lag value of the stimulation of the Zn emission band. With the available measuring equipment,† the stimulation of Zn emission as Fig. 3 shows, can actually be detected up to 10 min. after the action of the infra-red rays. The experiment is carried out as follows.

The SrSZnSm is fully excited, and then is immediately set for 5 sec. under the influence of a 40-w, 220-v vacuum tungsten filament lamp, working at 110 v, the light of which was filtered through a ruby red glass. Immediately after this, the measurement through suitable color filters was made, so that the emissions of Zn and Sm bands could be separated. Like the SrSZnBi, this SrSZnSm has remarkable afterglow duration and brightness as compared to the SrSZn and SrSSm. The difference in the behavior of the emission bands of Zn and Sm after a short action of infra-red rays leads to the conclusion that we have two different luminescence processes. If the electrons returned to the centers Zn and Sm without discrimination then the infra-red rays could influence all the emission bands in the same way, for example, they would stimulate all the bands.

Beyond this, we can conclude that the infra-red rays cause the transfer of electrons from the Sm traps to the Zn traps. This follows from the large time lag of the stimulation of Zn, that can only be explained if we assume that the number of electrons in the Zn traps augment under the action of the infra-red rays. This coincides very well with observed quenching of the Sm band. These ideas speak for a direct retrapping of electrons without ever having reached the energy of the conduction band.

[1] Urbach, Pearlman, and Hemmendinger, J. Opt. Soc. Am. **36**, 377 (1946).
[2] B. O'Brien, J. Opt. Soc. Am. **36**, 369 (1946).
[3] R. E. Ellickson, J. Opt. Soc. Am. **36**, 264 (1946).
[4] G. R. Fonda, J. Opt. Soc. Am. **36**, 382 (1946).
[5] G. F. J. Garlick, *Luminescent Materials* (Oxford University Press, New York, 1949).
[6] D. S. Bersis, Praktika Akad. Athenon (to be published).
* The Sm used contained Gd traces.
† Photo-voltaic cell with a sensitive mirror galvanometer connected across it.

Preliminary Results on the Spin of U^{235}*

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February 17, 1950

THE spin of U^{235} has been investigated by Tolansky[1,2] and also by Anderson and White.[3,4] Tolansky reported a nuclear spin greater than 3/2 based on studies with natural uranium which contains only 0.71 percent odd mass isotopic abundance. Anderson and White reported results on two arc lines, 5915A and 6826A, from material which had been enhanced to about 64 percent U^{235}. Anomalous intensities and hyperfine intervals in their patterns made interpretation difficult but they concluded that the nuclear spin was either 5/2 or 7/2 and suggested that the value of 5/2 was more likely correct.

We have made preliminary studies, on enhanced material, of the hyperfine patterns of several lines originating in the singly ionized uranium atom and find that the spin value 5/2 fits the experimental data considerably better than does a spin value of 7/2, as shown in Fig. 1. The value of 3/2 is not acceptable. The

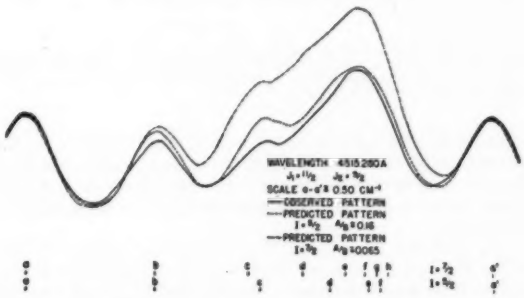

FIG. 1. Comparison of observed and predicted interference patterns of U II spectrum line. Observed pattern corresponds to average fringe intensity of four central orders of the Fabry-Perot pattern; predicted patterns are based on assumed negligible splitting in the upper level and assumed line shape and choice of A/B consistent with the observed pattern. Individual fringe patterns show greater detail.

anomalous intensity gradation and the breakdown of the Landé interval rule are readily explainable in terms of a quadrupole moment for the U^{235} nucleus. If the hyperfine energy is expressed as:[5]

$$T(\text{cm}^{-1})=\frac{A}{2}C+B\frac{\frac{3}{4}C(C+1)-\frac{1}{2}IJ(I+1)(J+1)}{IJ(2I-1)(2J-1)},$$

where $C=F(F+1)-I(I+1)-J(J+1)$, the best fit for the 4515A line is had for A/B set equal to approximately 0.16. A is proportional to the nuclear magnetic dipole moment, while B is proportional to the nuclear electric quadrupole moment. The hyperfine splitting is inverted and occurs mainly in the lower level $f^3ds(^6L_{\frac{11}{2}})$.

The experimental work is continuing and more complete results will be made available later.

It is a pleasure to acknowledge the assistance of J. M. Herndon in making available the enhanced U^{235}; of L. E. Burkhart who is in charge of the Isotope Physics Laboratory; of Miss L. M. Howard and E. C. Sienknecht for their contributions in reproducing the graph; and especially of P. M. Griffin for his invaluable contributions to the experimental phase of the work.

* This document is based on work performed under contract for the AEC by the Isotope Research and Production Division of the Carbide and Carbon Chemicals Division, Union Carbide and Carbon Corporation, Oak Ridge, Tennessee.
[1] S. Tolansky, BR-375, 1943 (available through USAEC, Technical Information Branch, Oak Ridge, Tennessee).
[2] S. Tolansky, BR-392, undated (available through USAEC TIB).
[3] O. E. Anderson and H. E. White, MDDC-333, September, 1946 (available through USAEC TIB).
[4] O. E. Anderson and H. E. White, Phys. Rev. 71, 911 L (1947).
[5] H. Casimir, Physica 2, 719 (1935).

Ricco's Law and the Quanta-Explanation of Vision

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February 13, 1950

RECENTLY[1] some measurements on the absolute thresholds of night myopia were reported. The authors found a decrease in the minimum quantity of light needed at the threshold when the night myopia is compensated. They employed visual angles ranging from 30 seconds to 10.5 minutes and retinal zones from the fovea up to 15° excentricity.

According to them the corrected eye needs at the threshold for the visual angles and the various spots of the retina studied about half the quantity of light energy compared with the uncorrected one. They suggest now that in our experiments[2] on the minimal number of quanta necessary for a light perception one quantum would have been the result when this phenomenon is considered, whereas we found two. We would like to discuss this suggestion in a few points.

Our conclusion on the minimum number of quanta is not founded on experiments dealing with the absolute number of quanta incident on the eye. The two-quanta explanation of van der Velden[2] is corroborated on investigations on the behavior of the threshold-value as a function of the time of observation and the visual angle of the test spot. The number two is quite defined by the law of Piper and the fact that the total number of quanta at the threshold as a function with the observation time t increases proportional to $t^{\frac{1}{2}}$. Out of Ricó's law and Bunsen's law it follows that these two quanta must be absorbed in two receptors within a certain area within a certain time. Even the wearing of tinted glasses does not influence this two-quanta conclusion.

Of course, in order to reach the threshold it is more efficient to converge a certain amount of light energy available in an area of the retina subtended by the region of visual angles within which Ricco's law is valid. Indeed, within such an area cooperation between the processes initiated by the absorbed quanta is possible: the processes of two absorptions result in a light perception.

So that, by correcting the myopia the illuminated area of the retina can be decreased and for small angular sizes of the test spot the threshold decreases, when the illuminated area of the retina becomes smaller than the area within which cooperation can occur. The general conclusion of Otero *et al.* about the decrease of threshold by correcting the myopia is explained by this fact. The visual angles used by the authors mentioned are in the region within which this phenomenon is important. The influence mentioned by the authors has a simple geometrically optical foundation and does not constitute a fundamental property of the way in which a light perception arises.

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[2] H. A. van der Velden, Physica 11, 179 (1944); Ophthalmologica 111, 321 (1946). M. A. Bouman and H. A. van der Velden, J. Opt. Soc. Am. 38, 570 (1948).

Transmittance of Interference Filters

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March 7, 1950

FIGURE 1 shows the transmittance of an interference filter, No. 7-1590-10, manufactured by Evaporated Metal Film Corporation for Baird Associates and described as having transmission bands at 4040 and 5910A, measured with polarized light having its electric vector perpendicular to the plane of incidence

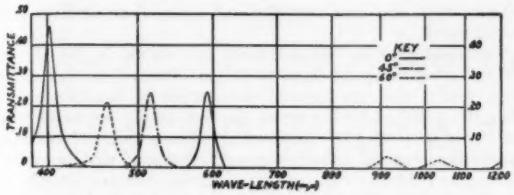

FIG. 1. Transmittance of polarized light having perpendicular electric vector at angles of 0°, 45°, and 60°.

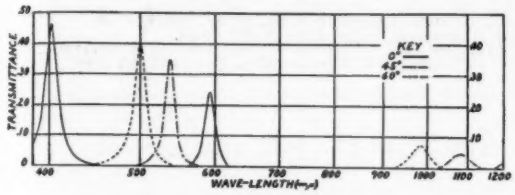

FIG. 2. Transmittance of polarized light having parallel electric vector at angles of 0°, 45°, and 60°.

and at angle of incidence of 0°, 45°, and 60°. Figure 2 shows the transmittance of the same filter at the same angles of incidence, but with the electric vector of the polarized light parallel to the plane of incidence. Figure 3 shows the transmittance of interference filter 7-1420-3, made by the same manufacturer and labeled as having a transmission band at 5545A, measured with random light at three angles of incidence.

These measurements were made in a modified General Electric spectrophotometer.

Figures 1 and 2 illustrate a use of retardation plate flickering in a General Electric spectrophotometer by which means it is possible to determine the transmittance of a specimen to polarized light of fixed orientation. Figure 3 illustrates a use of a retardation plate to produce the equivalent of random light for use in determining the transmittance of a specimen.

Figures 1 and 2 illustrate a use of the octaval (log wave-length) system of plotting spectrophotometric data in that the order of retardation can be determined easily. Each order of interference produces a transmission band. Assuming that the material between the metallic layers in the interference filter has a refractive index invariant with wave-length, the first-order band is separated from the second by 100 c.r.u. (100 $\log_2 2$), the first is separated from the third by 158 c.r.u. (100 $\log_2 3$) and the first from the fourth by 200 c.r.u. (100 $\log_2 4$) regardless of the exact wave-length positions.

In Fig. 1, the two bands in the visual range measured with normally incident light fall at 210 c.r.u. and 266 c.r.u. and are separated by 56 c.r.u. Thus they represent the second and third order of interference and a first-order band is expected at 376 c.r.u. The beginning of this band is just detectable with the Cetron 925 photo-tube used in the instrument. The discrepancy between the observed difference, 56 c.r.u. and the expected difference, 58 c.r.u. is caused by a slight increase in the refractive index of the dielectric at the shorter wave-lengths. This discrepancy is not large enough to introduce an uncertainty in the assignment of interference orders.

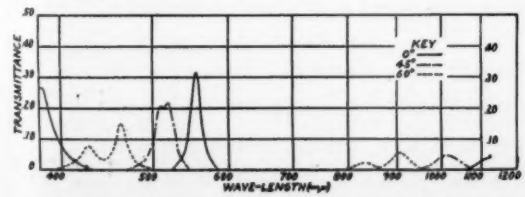

FIG. 3. Transmittance of random light at angles of 0°, 45°, and 60°.

Figure 3 illustrates the necessity for adhering strictly to normal incidence of light in order to produce "monochromatic" light at the wave-lengths designated by the manufacturer. On the other hand they also show the possibility of adjusting the "monochromatic" band to shorter wave-lengths by increasing the angle of incidence at the cost of widening the band.

These curves also show that by rotating a polarizing element in sequence with an interference filter at an angle to the incident light an alternate flickering of two adjacent "monochromatic" bands will be obtained. Such a light source would be useful in photometers used for evaluating the slope of an absorption curve.

[1] G. L. Buc and E. I. Stearns, J. Opt. Soc. Am. **35**, 521 (1945).
[2] W. A. Shurcliff, J. Opt. Soc. Am. **32**, 229 (1942).

Variation of Emission Spectrum of Manganese-Activated Zinc Beryllium Silicate with Decay Time

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March 22, 1950

IT has been stated by Fonda[1] that there is no color change during the decay of luminescence of manganese-activated zinc beryllium silicate, despite the fact that its emission spectrum consists of two well-separated bands, one in the green and one in the red.[2] In support of his statement he cites the results of Studer and Rosenbaum[3] who found the fluorescence and phosphorescence spectra of zinc beryllium silicate to be identical. Fonda concludes

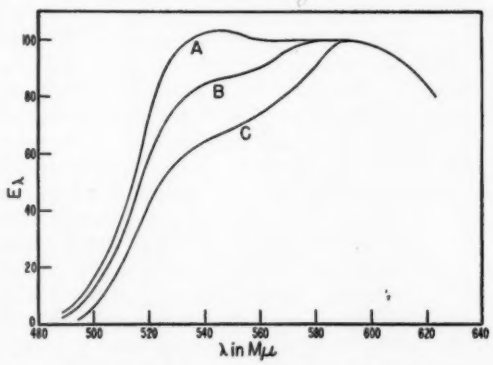

FIG. 1. Spectral emission of a $(Zn,Be)_2SiO_4$:Mn at different stages of its afterglow. Curve A: direct excitation; curve B: approximately 0.0002 sec. after removal at excitation; curve C: approximately 0.024 sec. after removal of excitation.

that this observation casts doubt upon the accepted view that the "green" and "red" centers in this phosphor are separate entities. He concludes further that the two emission processes, one giving green and the other red light, take place within the same center; and that both emission processes start from the same excited state, differing only in the energy levels to which the excited electrons fall. Fonda's statement and conclusions are at variance with the results of Kröger and Hoogenstraaten[4] and with other evidence that the "green" and "red" emissions are caused by different centers.

We have accordingly repeated the work of Studer and Rosenbaum, measuring the fluorescence and phosphorescence spectra

photoelectrically instead of photographically, as done by these investigators. For this purpose a 10-in. diameter aluminum disk was coated with $(Zn_{1.8}Be_{0.2})_2SiO_4$:1.5 wt. percent Mn in a 1-in.-wide band near its perimeter, and rotated at approximately 2300 r.p.m. Luminescence was excited by a small low pressure mercury discharge lamp, equipped with a Corning 9863 filter, placed close to the disk so that only a small segment of the phosphor circle was irradiated. Phosphorescent light was received by a monochromator pointed at another segment of the phosphor circle. By altering the position of the lamp, the angle between it and the monochromator could be varied, thus allowing examination of the phosphorescence spectrum at different decay times. The prism of the monochromator was motor-driven, the spectrum between 4000–8000A being scanned in 63 sec. The signal from the monochromator was received by a 1P21 photo-multiplier, whose output was then amplified and recorded on an Esterline-Angus meter.

The results of the measurements are shown in Fig. 1, E_λ for all curves being set equal to 100 at 5900A. It is clear from these results that the spectral distribution of the emission of $(Zn_{1.8}Be_{0.2})_2SiO_4$:1.5 wt. percent Mn *does* change appreciably as the decay proceeds. The differences we have observed between the fluorescence and phosphorescence spectra of zinc beryllium silicate are evidently too small to be readily detected visually or by the photographic method of Studer and Rosenbaum. Any conclusions based on the supposed invariance of the zinc beryllium silicate emission spectrum with decay time are therefore invalid.

[1] G. R. Fonda, J. Electrochem. Soc. **96**, 42C (1949).
[2] J. H. Schulman, J. App. Phys. **17**, 902 (1946).
[3] F. J. Studer and A. Rosenbaum, J. Opt. Soc. Am. **39**, 685 (1949).
[4] F. A. Kröger and W. Hoogenstraaten, Physica **15**, 557 (1949).